Summer 7-9-2004

Denitrification in Acid-Impacted Mountain Stream Sediments

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DENITRIFICATION IN ACID-IMPACTED MOUNTAIN STREAM SEDIMENTS

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Submitted to the Faculty of the Graduate School of the
University of Colorado at Boulder
In Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Summer 2004

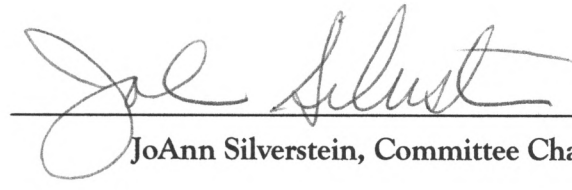
Department of Civil, Environmental, and Architectural Engineering

This thesis is entitled:

Denitrification in Acid-Impacted Mountain Stream Sediments

written by Jenny Lynn Baeseman

Has been approved for the
Department of Civil, Environmental, and Architectural Engineering



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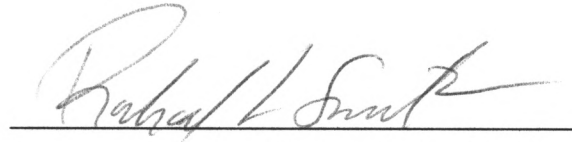

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Date: 7/09/04

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Baeseman, Jenny L. (Ph.D., Civil Engineering)

Denitrification in Acid-Impacted Mountain Stream Sediments

Dissertation Directed by Dr. JoAnn Silverstein and Dr. Richard L. Smith

Acid mine drainage (AMD) contaminates thousands of miles of mountain streams worldwide. At the same time, nitrate loading to many watersheds is increasing. Little is known about nitrogen cycling in acidic, heavy metal-laden streams, however it has been reported that denitrification, the reduction of nitrate to nitrogen gas, is inhibited under low pH. Snowmelt dominated mountain streams, bringing a pulse of nitrate, dissolved organic carbon, and in AMD sites iron, sulfate, heavy metals, and acidity.

The objectives of this research were to determine if denitrifying microorganisms are present and active in AMD streams, investigate the effects of pH, electron donors, and iron on denitrification in acidic and circumneutral streams, examine seasonal affects on denitrification, and compare microbial diversity in AMD streams with that in a naturally acidic stream and a pristine mountain stream.

Nitrogen gas was the major end-product of denitrification in sediments from several AMD impacted streams in Colorado with pHs ranging from 2.60 to 4.54, with no correlation between rate and initial pH. Denitrification increased pH in all microcosms. Carbon stimulated denitrification, as well as the presence of high DOC in stream waters, showing the importance of heterotrophic denitrifiers. Additional soluble iron (Fe^{2+} and Fe^{3+}) decreased denitrification. Denitrifiers were present and active throughout the year in sediments from a direct mine effluent stream, a higher-order AMD-impacted stream, a naturally acidic stream, and a pristine, near-neutral pH mountain stream. Stream nitrous oxide concentrations correlated with highest denitrification potential rates during snow cover indicating microcosm studies complemented in situ denitrification activity.

rRNA genes amplified from environmental DNA included members from all three domains and 21 different bacterial divisions and 715 previously unidentified strains. No common denitrifiers were found, however rate constants significantly correlated with the number of clones found in *α-Proteobacteria*, *Actinobacteria*, and *Chloroflexi*, indicating that denitrification may be widespread within these groups.

Denitrifiers are active in acid-impacted streams and are influenced by environmental and hydrologic conditions. The results presented here have increased our understanding of nitrogen cycling in extreme environments as well as environments were multiple stressors are present and may be important for nitrogen and carbon budgets in mountain catchments.

Keywords: denitrification, acid mine drainage, microbial diversity

Dedicated to Charlie,
wherever you are

ACKNOWLEDGEMENTS

This dissertation would not have been possible if it weren't for the efforts of my advisors, Dr. Richard L. Smith and Dr. JoAnn Silverstein. Thank you for four years of encouragement and mentorship. I look forward to working with you more in the future. I would also like to thank my committee members, Dr. Norman Pace, Dr. Mark Hernandez and Dr. Diane McKnight, for their insightful comments in designing this research.

Thanks to the National Science Foundation and the U.S.G.S Water Resources Division for funding for this research.

I also owe a debt of gratitude to Jeff Walker for his help with the microbial genetics work. Your patience, understanding, and ability to explain difficult concepts to an 'engineering' was remarkable. Thank you, not only for all the effort you put in to guiding this rather large chunk of sequences, but for the laughs. Kirk and John, I always looked forward to seeing your smiling faces in the lab and enjoyed all the fun and thought provoking conversations. Dan, thanks for all your help with XplorSeq, and for letting me use it, a truly wonderful concept!

A special thank you goes to all who have helped me with sampling efforts and analysis. Deb, thanks for always being there with the answers and for your friendship. Charles, I imagine after my samples, you never want to do another dilution again. Thanks for it all, and for all the wonderful Antarctic conversations! Andrew Todd, thanks for driving the snowmobile on a 45° slope and for calming me down after we 'tipped', and of course for pulling me out of the snow the myriad of times I feel in. Christopher Jaros, you are truly an ATV master, not only on the Ice, but in the forests of Montezuma as well. I hope you enjoy your photo in Chapter 3. Thanks for everything. Mike Ryman, thanks for tagging along with me in the field so many times and for all the alphabet games we used to play. I still owe you a beer. Ben Heavner, working with you was a true joy. Tricia Renton, thanks for all your help with the preliminary work that set the basis for this dissertation, but more so, thank you for making my mentoring experience such a pleasant one. Amani McHugh, thanks for

teaching me that not everyone is meant for research. Kurt, your help with all the DOC analyzes is greatly appreciated. Blaine McCleskey, thank you for all your help with the metal analysis, and for teaching me the famous iron speciation analysis technique. Natalie, thanks for asking me for my notes in Environmental Microbiology, for getting me out, for all your help with the DOC samples, and for making me take a belly dancing class! Joy, Azra, and April, thanks for all your help, encouragement and in-depth discussion about grad school, and most importantly your friendship. Kim, thanks for being another cool Cheesehead; your help and friendship mean a lot, as does your belly dancing.

Diane McKnight, thank you for giving me the opportunity of a lifetime, and a much needed break from reality. My Antarctic experience changed my life in so many ways, and each day that goes by I realize just how important that 'trip' was for me. I'm hooked. Thank you for taking a chance on me and letting me head "Algae Ops". To Gregg and Steve, for surviving the crash and teaching me just what "the spirit to live" really means; you are both an inspiration to me and I will always be grateful we met, even under such difficult circumstances. To Lemon Head, Krispy Kreme, Juicy Grapefruit, Porn Star, and EnVogue: I will never be able to listen to Pink without thinking of you guys and how great F6 was. Thank you for making the experience of a lifetime so special. Pepperoni, thanks for making lasagna in a box. Juicy, 'Endangered species are just charismatic megafauna from a cush ecozone to a resident extremophile'. To 'Brenda Hall's Group and the Kiwis, Taco Nacho, the Yeastie Boyz, Ice-freezing guy, Packer Doug, Diver Doug, Snake Bar, the Wind-Erosion Guys, Laura, Gifford, Brian, Chris, Scotty, Remediation Guy, Don Juan, and countless others for listening to my explanations of acid mine drainage and denitrification and how that got me to the Ice. Homer Simpson, thank you for your beer song. A special thank you to the Bearded Joker, thanks for the morning wake-up calls and for teaching me a thing or two. I'll be back.

I would like to acknowledge a few of the people who were influential in getting me to this point: Wayne Kleinschmidt, Bryant Browne, Earl Spangenberg, Paige Novak, Joe Ryan, and Jim Hurley. Thanks for taking a chance on me. And to my

teachers, you shaped my mind in so many ways, thanks for getting through this thick skull, and making me believe that I could do whatever I set my mind to.

To all my friends and loved ones, your encouragement and support over the past 22 years are priceless. I especially want to thank Jason, Train, Ray, the Houfe's (all of you), Louise, Chief, Cheezy, Foster, Jeremy, Toni, Val, Lacy, Erin, Barry, Gregg, Gifford, Joy, Mindy, Karen, Natalie, Sarahann, Chris, Ben, Neal, Jamie, Brian, Shawn, Chad, Brenda, Lori, Kevin, Andrew, Grant, Ben, Vicki, Eric, Randy, Mike, Tracy, Joanne, Penny, Connie, Mark, Denice, Juan, Tami, and Vegas; you continually believe in me and who I am, your 'reality checks' and 'Baeseman, I don't know how you do it' and 'I can't believe you are still in school' kept me going. I have been blessed with the greatest friends and support network, thank you all. And to Tumor, thanks giving me so many great stories to tell.

I would like to send out a special thanks to HP for making reliable printers, to whoever figured out that coffee beans are the greatest thing (even better than sliced bread), to Ben Franklin for inventing electricity, to Bill Gates and Steve Jobs for making a students life easier, to Al Gore for the Internet and last but certainly not least, to Jack Daniels.

A special thank you to my family for finally accepting that I am not going to be a farmer ☺. And to my nephew, Nathaniel, thank you for reminding me that learning is fun.

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CHAPTER 1: INTRODUCTION

Acid mine drainage (AMD) is a major problem in many parts of the United States, as well as worldwide. The U.S. Environmental Protection Agency estimates that more than 7,000 km of streams are impacted by AMD from coal mining in the eastern United States alone [EPA 1994]. The U.S. Forest Service projects that between 20,000 and 50,000 mines are actively generating acid on forest service lands in the western United States, contaminating between 8,000 and 16,000 km of stream reach [USFS 1993]. Many of the mines generating acid are abandoned, and the costly remediation of these sites often falls under governmental responsibility.

AMD is characterized by extremely low pH, high heavy metals and often high sulfate concentrations [USFS 1993]. During the mining process, rocks are brought to the surface, precious metals are removed, and leftover waste rock, called *tailings*, are piled on the surface. As snowmelt and other precipitation events infiltrate these piles, pyrite and other sulfidic minerals contained in many of the rocks are chemically oxidized, which generates iron and sulfuric acid. The presence of microorganisms in the tailings has been shown to greatly increase this weathering [Edwards et al. 1999]. The sulfuric acid causes many other metals to solubilize, and the resulting low-pH, heavy-metal waste leaches into nearby streams, creating an extreme environment for life [Madigan 2000].

Some research has been conducted on microbiological diversity in AMD environments. Diversity studies have focused mainly on the iron- and sulfur-cycling

organisms living in the tailings and in the direct mine effluent rather than on the downstream impacts of AMD [Bond and Banfield 2001, Edwards et al. 2000, Fortin 1996, Marchand 2000]. Studies using 16S rRNA techniques to assess the microbial community show relatively limited diversity in mine-effluent streams [Bond and Banfield 2001, Edwards et al. 2000]. Research has also shown a decrease in both the diversity and number of invertebrates that can survive in these environments, as well as the impact on higher organisms, such as fish [Niyogi et al. 1999]. In St. Kevin Gulch near Leadville, Colorado, it was found that 99% of the biomass could be attributed to one green algal species, *Ulothrix* [Niyogi et al. 1999]. The low pH and high concentration of dissolved metals in this stream were toxic to periphyton and other organisms living on or in the sediment.

Most of the research in AMD-impacted streams has focused on heavy metals, particularly iron. Information on other biogeochemical cycling in AMD streams is limited mainly to dissolved organic carbon (DOC). Studies by McKnight and others showed that fulvic acid, which constitutes about 50% of the total DOC, can be sorbed onto iron oxyhydroxides present in acid-impacted environments [Boyer et al. 2000, McKnight et al. 2002]. They suggested that the fulvic acid could be important to stream diversity because it may limit in-stream heterotrophic activity or because the sorbed DOC may be available for sediment microbial uptake [McKnight et al. 2002]. Carbon and nitrogen are both important constituents in a stream ecosystem. Although nitrogen is a requirement for all life [Madigan 2000], there is very little information on the availability and cycling of nitrogen in AMD-impacted streams. The presence of

algal mats and slimes in these extreme environments demonstrates that nitrogen is available in some form to support life. However, reports on nitrogen species and abundance are sparse. Perhaps the two most important processes involving nitrogen in these environments are nitrogen fixation, which introduces fixed nitrogen into the stream ecosystem, and denitrification, the reduction of nitrate to nitrogen gas, which is a primary removal mechanism for nitrate, the principal form of fixed inorganic nitrogen in an oxidizing environment.

Denitrification has shown to be inhibited by low pH in forest soils [Blosl and Conrad 1992, Christensen 1985, Nagele and Conrad 1990]. Limited studies have investigated the effects of certain heavy metals on denitrification rates, with results showing that some metals stimulated the process whereas others hindered metabolic activity [Iwasaki and Terai 1982, Slater and Capone 1984]. It is also likely that the electron-donor supply affects denitrification in AMD environments, based on previous studies in this setting [Johnson 1998]. Studies on denitrification in groundwater could be relevant to AMD environments because many groundwater systems are also electron donor-limited. Denitrification in some organic donor-limited systems can be autotrophic, meaning iron, hydrogen and other alternative electron donors are used for reducing nitrate to nitrogen gas [Chapelle 2001].

The research presented here focuses on denitrification as a primary mechanism for nitrogen loss in acidic, heavy metal-laden streams. Atmospheric nitrogen deposition has been shown to be of increasing concern in some areas of the Rocky Mountains because of the limited capacity for absorption of nitrogen in many alpine

regions, leading to the export of nitrate in streams and groundwater flow [Williams 2000]. One objective of this research was to examine if these already impacted AMD streams could process increased loads of nitrogen, particularly in the form of nitrate. Denitrification, the biological conversion of nitrate to nitrogen gas, may be very important in these systems if they receive increased nitrogen through atmospheric deposition. In addition, denitrification generates alkalinity, and enhanced nitrate reduction could raise the pH of these systems, reversing the impacts of AMD on biota. Finally, because of limited knowledge of nitrogen species in AMD systems, the possibility that nitrogen is limiting microbial activity in these streams was also examined by the addition of nitrate to sediment samples.

This dissertation research is relevant to both basic and applied stream ecology and nutrient cycling. Because these AMD-impacted mountain streams are greatly influenced by periods of snow cover, snowmelt and low-flow summer conditions, the affect of seasonal conditions on water quality and denitrification was investigated. In addition, to better understand if AMD streams can be restored to natural conditions, the microbial community, structure and diversity in AMD streams was compared with that of a pristine mountain stream and of a naturally acidic stream.

Objectives

The objectives for this research were to: (1) determine if denitrifying microorganisms are present and active in AMD-impacted streams, (2) investigate the effects of pH, available electron donors and iron on denitrification potential in water and sediments from acidic and circumneutral streams, (3) compare the impact of water chemistry on denitrification potential during snow cover, snowmelt and summer flow conditions, and (4) compare microbial diversity in AMD streams with that in a naturally acidic stream and in a pristine mountain stream.

Organization

This dissertation is divided into seven chapters. Chapter 2 provides a comprehensive literature review of relevant topics. The descriptions of the sites used for this study, as well as the detailed methods used, are included in Chapter 3. Chapter 4 presents results of microcosm incubations from several AMD-impacted streams and the effects of added potential electron donors, pH and iron on denitrification. Chapter 5 provides an analysis of the variation of denitrification rate potential and water chemistry during snow cover, snowmelt and summer flow conditions in two streams affected by AMD, a naturally acidic stream and, for comparison, a pristine mountain stream. The microbial diversity in these same sites during the three flow conditions is discussed in Chapter 6. Specific and general unifying conclusions from this research, as well as suggestions for future research, are presented in Chapter 7.

CHAPTER 2: LITERATURE REVIEW

Environmental contamination is of great concern, not only in the United States but worldwide. Because of the extent of contamination, an increasing trend is the interaction of more than one type of contamination. Little is known about environments where several types of pollution occur. In mountain watersheds, two interacting sources of contamination are acid mine drainage (AMD) and atmospheric nitrogen deposition; until now, there had been no research on nitrogen cycling in AMD environments. This chapter provides an overview of the literature pertinent to understanding the biogeochemistry of nitrogen, particularly the removal of nitrate from AMD-impacted soils and streams.

The Nitrogen Cycle

In nature, the nitrogen cycle is complex and has many intermediate species (Figure 2-1). Because some of the key reactions in this cycle are carried out exclusively by microorganisms, the activity of those microorganisms in the environment is crucial. Nitrogen fixation, ammonification, nitrification and denitrification are the main processes in the nitrogen cycle.

Nitrogen gas, N_2, is the most thermodynamically stable form of nitrogen and compromises about 78% of Earth's atmosphere. Nitrogen fixation is the biological reduction of N_2 to ammonia (NH_3), which is then assimilated into organic-N and used for cell function. Breaking the triple bond in N_2 is a high energy–demanding process,

and several microorganisms that fix nitrogen have been characterized. Genera include *Azotobacter*, *Cyanobacteria*, *Clostridium*, *Rhizobium*, *Bradyrhizobium* and *Frankia*, among others (see [Madigan 2000] for review).

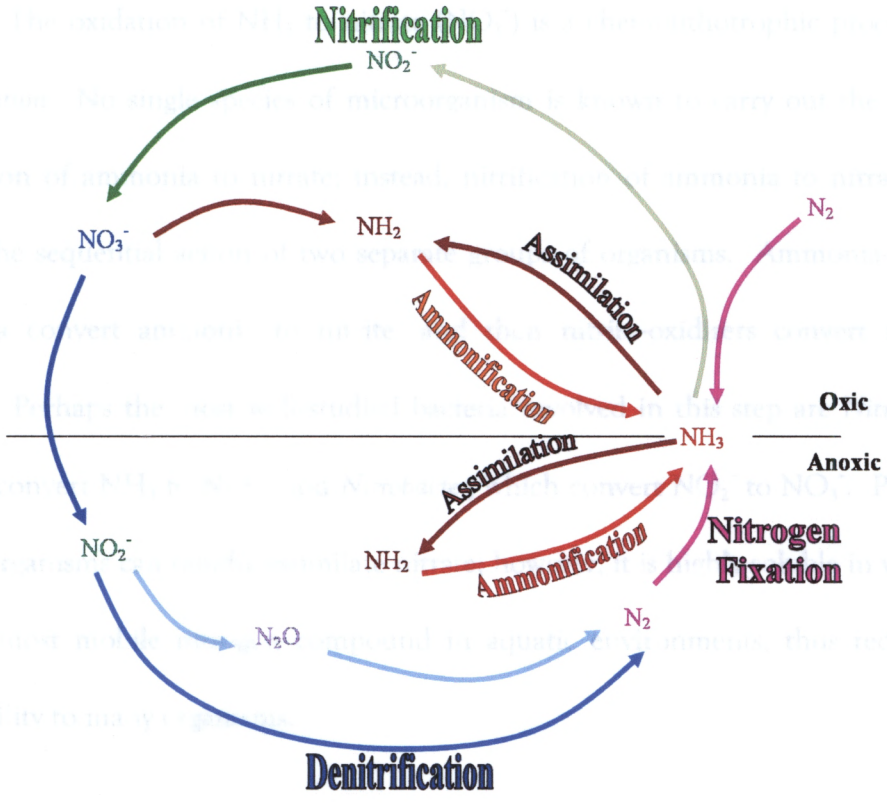

Figure 2-1. The Nitrogen Cycle
Adapted from *Brock Biology of Microorganisms* [Madigan 2000]

Ammonification is the decomposition of organic N to ammonia, which is usually present in circumneutral aquatic systems as the ammonium ion (NH_4^+). Ammonium, the stable redox state under anoxic conditions, is the soluble nitrogen species that dominates in most anoxic sediments because it is cationic and sorbs to soil particles. Ammonia is rapidly recycled and converted to amino acids by plants and microorganisms in soils. Because deprotonated ammonia (NH_3) is volatile, some loss

can occur from soils, particularly highly alkaline soils; volatilization is a major contributor to the atmospheric ammonia in areas with dense animal populations.

The oxidation of NH_3 to nitrate (NO_3^-) is a chemolithotrophic process called *nitrification.* No single species of microorganism is known to carry out the complete oxidation of ammonia to nitrate; instead, nitrification of ammonia to nitrate results from the sequential action of two separate groups of organisms. Ammonia-oxidizing bacteria convert ammonia to nitrite, and then nitrite-oxidizers convert nitrite to nitrate. Perhaps the most well-studied bacteria involved in this step are *Nitrosomonas,* which convert NH_3 to NO_2^-, and *Nitrobacter,* which convert NO_2^- to NO_3^-. Plants and microorganisms can rapidly assimilate nitrate; however, it is highly soluble in water and is the most mobile nitrogen compound in aquatic environments, thus reducing its availability to many organisms.

Removal of Nitrogen from Aquatic Environments: Nitrate Reduction

In the environment, there are six known processes through which nitrate can be reduced: assimilatory nitrate reduction to ammonium, dissimilatory respiratory denitrification, dissimilatory nitrate reduction to ammonium, nitrate respiration, non-respiratory denitrification and chemodenitrification (see [Tiedje 1994] for review). Nitrate can be reduced to ammonium by many bacteria (assimilatory nitrate reduction). The ammonium is then incorporated into the cell biomass. This process is inhibited by low concentrations of ammonium or organic nitrogen in surrounding

environments, probably because ammonia is the energetically favorable nitrogen source for cell synthesis (see [Zumft 1997] for review). This process is not expected to occur in anaerobic environments where ammonium and organic nitrogen are usually in high concentration.

The use of nitrate as an electron acceptor by bacterial respiration is quantitatively the most important to fixed nitrogen budgets [Driscoll et al. 2003]. The definitive feature of this process is the production of nitrogen gases, N_2O and N_2, from nitrate and nitrite, coupled with growth yield (Equation 1). In this process, there is a rapid production of gaseous products, with a nearly stoichiometric conversion of nitrate or nitrite to N_2O or N_2, characterized by the presence of dissimilatory nitrate, nitrite, nitric oxide or nitrous oxide reductase enzymes (see [Zumft 1997] for review).

$$NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$$

Equation 1. The Denitrification Pathway

The nitrate reduction process is not always carried completely to nitrogen gases. Nitrate-respiring bacteria are not "true" denitrifiers, in that they reduce nitrate to nitrite and cannot use nitrite or other reduced nitrogen oxides as electron acceptors, resulting in an accumulation of nitrite. It has been suggested that these bacteria may have a selective advantage over other bacteria in a mixed culture because the cell yield for nitrate reduction is higher than for denitrification using nitrite as the electron donor [Turk and Mavinivc 1987]. The accumulation can also occur when electron donors, such as carbon, are limited [Oh and Silverstein 1999, VanRijn et al. 1996]. In a study on denitrification in activated sludge, the process was inhibited by the

accumulation of nitrite, which the authors attributed to the accumulation of HNO_2 [Glass et al. 1997], which is extremely toxic [Abeling and Seyfried 1992]. At low pH, such as in acid-impacted mountain streams, the toxic effect of nitrite on denitrification may be important.

A few studies have shown nitrate reduction in the presence of oxygen [Robertson and Kuenen 1984]; however, it is widely accepted that denitrification is mainly an anaerobic respiration process. Oh and Silverstein showed that denitrification rates were reduced by as much as 50% in the presence of less than 1 mg/L dissolved oxygen [Oh and Silverstein 1999]. A few microorganisms have been found that are capable of denitrification in the presence of oxygen. Thomas et al. showed that several species of *Pseudomonas aeruginosa*, PAO1 and isolate 7, were capable of denitrification under aerobic conditions after growth under both aerobic and anaerobic conditions [Thomas 1994]. However, *P. fluorescens* was not capable of denitrification under aerobic conditions. If denitrifying organisms are present in AMD streams, it is likely that they are found in anoxic zones or microenvironments. Some researchers suggested that pockets of limited oxygen may exist in biofilms on rock surfaces and in slimes that are commonly formed in AMD environments [Bond et al. 2000].

Non-respiratory denitrification results primarily in the production of N_2O from nitrate or nitrite, with no enhanced growth [Tiedje 1994]. Only 1% to 10% of the nitrate or nitrite reduced by these organisms ends up as N_2O. Nitrate-assimilating bacteria are capable of this process, as are yeasts, filamentous fungi and algae. The

mechanisms of N_2O production are variable and may result from nonspecific reactions by metallo-enzymes, such as cytochromes P-450 [Shoun and Tanimoto 1991].

Possible Factors Affecting Denitrification in Acid-impacted Systems

Effects of Low pH on Denitrification

In soils with pH below 5, chemodenitrification may be of particular importance [Horio et al. 1981]. This process is abiotic and involves the reduction of nitrite to predominately NO, but N_2O and N_2 have been reported. In one laboratory study, nitrite (NO_2^-) was abiotically transformed to nitrous oxide around pH 2 [Appelo and Postma 1993]. Chemodenitrification is not thought to be of global importance, but there are areas, such as acidic forests, salting out in frozen soils and wetting of dry soils, where it may be significant. It is not clear whether this process accounts for nitrate transformation in acid-impacted stream ecosystems. Nitrite tends to be an environmentally unstable form of nitrogen, and there is little data about the presence of nitrite in acid environments.

Studies involving the biological denitrification process in acid environments have focused on acidic bogs and forest soils. Christensen stated that virtually no N_2 is formed in acidic soils below pH 5 [Christensen 1985]. The main product of denitrification in acidic soils has been reported to be N_2O. In studies by Conrad and co-workers, organisms capable of utilizing nitrate were mostly nitrate respirers [Blosl and Conrad 1992, Nagele and Conrad 1990a, 1990b]. When the pH of the acidic forest soil used was increased to 7, a shift from predominately nitrate respirers to a

more diverse group consisting of nitrate-respiring organisms, a small community of nitrate ammonifiers and some denitrifiers was observed. They also demonstrated that in an acidic soil, nitrate-reducing potentials decreased with decreasing pH, and the proportion of NO and N_2O produced from nitrate reduction increased. When the acidic soil's pH was raised to 7, less NO and N_2O were produced. When an alkaline soil with pH 7.8 was acidified to a pH of 4, NO and N_2O were no longer produced, and denitrification stopped. The authors suggested that this resulted from increased concentrations of toxic aluminum and that the microbes in the alkaline soil were incapable of adapting to the acidic conditions, whereas the microbes present in the acidic soil had already adjusted to those conditions.

Parkin and co-workers isolated denitrifiers with a pH tolerance range from 2.9 to 4.8, with an optimum pH of 3.9 [Parkin 1985]. However, the denitrification rates of these isolates were lower than those of denitrifiers found in a nearby neutral pH soil. Glass and Silverstein attributed the inhibition of denitrification at low pH to the accumulation of nitrite in a denitrifying activated sludge culture [Glass and Silverstein 1998]—nitrite in high concentrations can be toxic to denitrifiers. Abeling and Seyfried [1992] found that nitrous acid (HNO_2) was the most toxic species to denitrifiers, inhibiting activity at a concentration as low as 0.04 mg/L HNO_2-N. HNO_2 is a weak acid with a pKa of 3.7 [Lange's Handbook of Chemistry 1987]. At pH values near 3.7, nitrite concentrations as low as 0.1 mg/L of nitrogen could be toxic to denitrifying bacteria [Abeling and Seyfried 1992]. Glass and Silverstein observed that nitrite was produced in almost stoichiometric amounts at high pH [Glass and Silverstein 1998].

Research on abiotic nitrate and nitrite chemistry has also been conducted. Under basic conditions (pH>8), nitrate can be abiotically reduced to ammonium or nitrite in the presence of Fe^{2+} and to nitrogen gas in the presence of aluminum [Fanning 2000]. Nitrate has also been shown to be abiotically reduced to ammonium in the presence of green rust (Fe(II, III) hydroxides) [Chr et al. 1996], where no nitrite, an intermediate in the denitrification pathway, appeared. Nitrite can be very reactive at low pH [Bollag et al. 1973], particularly in the presence of Fe(III) and light [Zhang and Bartlett 2000]. Nitrite can be oxidized to NO_2 (gas) by free hydroxyl radicals from the photolysis of $FeOH^{2+}$, a form of Fe(III) [Zhang and Bartlett 2000].

Effects of Metals on Denitrification

A limited number of studies have been done on the effects of metals on denitrification. Iwasaki and Terai grew two denitrifiers, one in the presence of copper and the other in copper-free media [Iwasaki and Terai 1982]. *Alcaligenes faecalis* was grown in media with 0.2% KNO_3 and 1.1 µM $CuCl_2$, and *Alcaligenes* sp. IAM 1015 was grown in 0.2% KNO_3 and 5.5 µM $CuCl_2$. When the denitrifiers were grown in copper-depleted media, N_2O was the predominant end product, with little accumulation of N_2. In the media containing copper, little to no N_2O was detected, and the organisms were capable of complete denitrification to N_2. The authors concluded that copper was necessary for denitrification. Slater and Capone investigated the affects of $HgCl_2$, $PbCl_2$, $NiCl_2$, $CuCl_2$, $FeCl_3$, $CdCl_2$, $K_2Cr_2O_7$, K_2CrO_4, Na_2MoO_4 and $ZnSO_4$ on denitrification in anoxic salt marsh sediment at 1000 ppm

(weight metal: weight dry sediment) [Slater and Capone 1984]. The presence of most of the metals examined (Hg, Pb, Ni, Cr, Zn, Cu, Fe and Cd) caused an initial lag in nitrous oxide production, except for Mo, which produced no lag. Cr, Pb and Mo stimulated total denitrification rates. Zn and Cu appeared to stimulate denitrification, but results were not statistically significant. Ni depressed the maximum amount of nitrous oxide produced overall. Fe seemed to decrease denitrification; however, the results were not statistically significant. These results are important to consider for denitrification potential in AMD sediments, where elevated concentrations of soluble metal ions and complexes are likely to be present.

Alternative Electron Donors for Denitrification

Denitrification is generally carried out by heterotrophic bacteria. Thus, another environmental constraint on denitrification is the availability of electron donors. Studies suggest that carbon in AMD streams is limited [Chapelle 2001, Johnson 1998]. The seasonality of carbon fluxes into AMD streams may also affect the availability of carbon and energy substrate to the heterotrophic microbial community. After leaf litter enters the stream and is degraded, a pulse of organic electron donors may become available in the AMD stream ecosystem and may enhance heterotrophic processes. This, in turn, may cause a seasonal shift in the heterotrophic microbial community structure and increase the ability of heterotrophic denitrifiers to reduce nitrate [Allan 2000]. Heterotrophic denitrification may be particularly important in AMD

environments because it can generate alkalinity, which may raise the pH of surrounding waters [Stumm and Morgan 1996].

Autotrophic metabolic processes may also be important in the cycling of nitrogen in AMD streams. In fact, the availability of alternative electron donors may be especially important in these carbon-limited systems. Researchers have shown that elemental sulfur can be an electron donor in the denitrification process [Devlin 2000, Jenneman 1986, Yamamoto-Ikemoto 2000]. In a study on material from a nitrate-contaminated aquifer in Canada, acetate, hydrogen gas, elemental sulfur, thiosulfate, aqueous ferrous iron and (solid) pyrite were tested as potential electron donors in the denitrification process [Devlin 2000]. All these electron donors stimulated nitrate removal except pyrite. Granular iron (Fe^0) was also used as an electron donor in this study and was found to reduce nitrate to ammonium at neutral pH with pyrite as a buffer. A mixed, granular, iron aquifer sediment containing several electron donors (hydrogen, ferrous iron and pyrite) supported denitrification; however, the abiotic reaction with granular iron dominated, and ammonium was the major end product.

Studies have shown that Fe^{2+} is a potential electron donor in the conversion of nitrate [Devlin 2000, Schuler 1999, Till 1998]. Fe^{2+} is released during the oxidation of pyrite and could be a potential electron donor in AMD environments. The theoretical stoichiometric reaction for nitrate reduction via Fe^{2+} is shown in Equation 2.

$$10Fe^{2+} + 12H^+ + 2NO_3^- \rightarrow 10Fe^{3+} + N_2 + 6H_2O$$

Equation 2. Stoichiometric equation for nitrate reduction with Fe^{2+}

The abundance of metals, particularly iron, and of sulfate in AMD streams may create conditions for biogeochemical nitrogen-cycling processes not seen in circumneutral waters. Chaudhuri and co-workers explored the importance of nitrate in the biogenesis of magnetite [Schuler 1999]. This research showed that the anaerobic bacterium *Dechlorosoma suillum* strain PS uses nitrate as the electron acceptor to form greenish-gray mixed Fe^{2+}-Fe^{3+} hydroxides, known as carbonate-containing green rusts. These rusts are unstable in the environment, and further oxidation leads to the formation of magnetite. Hansen and co-workers [1996] also demonstrated the formation of green rust in the presence of nitrate. Although green rust can form without the presence of nitrate, this research demonstrated that the nitrate present was reduced to ammonium, not N_2, under abiotic conditions at a pH of 8.25 at 25°C. The formation of green rusts in abiotic conditions could also lead to the formation of magnetite if oxidizing conditions exist. The presence of high quantities of Fe^{2+} and Fe^{3+} and nitrate in AMD streams may be important factors in the generation of green rust and, eventually, magnetite, because of the oxidizing environment. The results of the magnetite formation reaction would include removal of nitrogen from water, iron precipitation and bacterial growth.

Another inorganic electron donor is hydrogen, which heterotrophic denitrifiers can use as an energy source. *Paracoccus denitrificans* can use hydrogen for energy while reducing nitrate–a reaction shown in Equation 3 [Devlin 2000]. Organisms that are capable of this mixotrophic denitrification process are not chemolithotrophic, because they lack Calvin-Benson cycle enzymes for fixing CO_2 and, so, require organic carbon

for cell synthesis. Through this process, one mole of hydroxide is generated per mole of nitrate reduced to N_2. This process may serve as a source of alkalinity, which may be important in buffering AMD environments. However, it is not known how much hydrogen may be available in AMD streams.

$$2NO_3^- + 5H_2 \rightarrow N_2 + 4H_2O + 2OH^-$$

Equation 3. Denitrification with hydrogen as the electron donor.

Elemental iron may serve as a source of hydrogen in these environments. When elemental iron is immersed in water, its oxidation is coupled with the reduction of water-derived protons [Till 1998]. This reaction (Equation 4) produces hydrogen and Fe^{2+}.

$$Fe(0) + 2H_2O \rightarrow H_2 + Fe^{2+} + 2OH^-$$

Equation 4. Hydrogen generation by the oxidation of elemental iron

Research at the University of Iowa has shown that autotrophic or mixotrophic denitrification, with hydrogen as the electron donor, is greatly accelerated by the presence of elemental iron [Till 1998]. In AMD environments, elemental iron could participate in the acceleration of denitrification. Till [1998] showed that denitrification with hydrogen and elemental iron occurred at pH values ranging from 5 to 9. At the lowest pH of 5, a lag period preceded denitrification, which could be explained by the time it takes for the species used, *Paracoccus denitrificans*, to adapt to the lower pH. This adaptation may be important when studying denitrification at lower pH. It is possible

that other species of denitrifiers can adapt to, or may even require, acidic conditions, which may be important in AMD environments.

Moura et al. [1997] showed that sulfate-reducing bacteria can also reduce nitrate. *Desulfovibrio desulfuricans* ATCC 27774 induces nitrate reductase, the enzyme responsible for conversion of nitrate to nitrite, and a type nitrite reductase, which reduces nitrite to ammonia. *D. desulfuricans* reduces nitrate, but has not been shown to denitrify. The coupled reaction of sulfate reduction with the conversion of nitrate to nitrite to ammonia has a net energy yield for the cell. The presence of nitrate actually increases the rate of sulfate reduction for this bacteria, as well as for *Desulfovicrio gigas* [Moura et. al 1997].

Nitrogen in Mountain Ecosystems

Studies have shown that nitrogen deposition is increasing in the Rocky Mountain Front Range and has resulted in elevated levels of nitrate in many alpine streams [Williams 2000, Williams 1996a]. The streams originating in the Rocky Mountains provide much of the drinking and irrigation water for the intermountain west and much of the Great Plains. Sparse vegetation and soil cover in high mountain catchments result in reduced nitrogen assimilation capacity; thus, these areas are particularly sensitive to pollution from atmospheric nitrogen deposition as compared with the lowland regions.

Nitrogen deposition from atmospheric pollution is an increasing problem in many parts of the world [Williams 1996a]. Of particular concern is the addition of

excess nitrogen to environments that may not be capable of assimilating the increasing nitrogen loads. Alpine and subalpine regions in the Rocky Mountains have been recognized as an example of such areas because of the lack of necessary vegetation to assimilate deposited nitrogen [Williams 2000]. Nitrogen saturation in these regions has become a major concern in recent years. Excess nitrogen, usually exported as nitrate to surface water, can cause algal blooms and eutrophication and can lead to decreasing species diversity [Williams 2000]. In addition, the presence of nitrate in drinking water has been linked to gastric cancers and causes blue baby syndrome in infants [Williams 1996b].

Historically, significant mining activity occurred in high mountain regions of the western United States during the 19th and 20th centuries. Exposure of waste rock and tailings containing pyrite (FeS_2) to snowmelt and oxygen led to the formation of acid mine drainage, which is discharged into mountain streams. The increasing amounts of nitrogen entering alpine streams, coupled with the number of these streams impacted by AMD, lead to the question: What is happening to nitrogen loads in low pH environments? However, there is limited information available on the behavior of nitrogen species in acidic environments. Because most of the nitrogen in atmospheric deposition enters water bodies as nitrate, the methods by which nitrate is removed from aquatic environments warrants particular investigation.

Acid Mine Drainage

Acid mine drainage (AMD) is a major problem in both the eastern and the western United States, as well as worldwide. The EPA estimates that more than 7,000 km of streams are affected by AMD from coal mining in the eastern United States [EPA 1994]. The U.S. Forest Service estimates that between 20,000 and 50,000 mines are actively generating acid on forest service land in the western United States, contaminating 8,000 to 16,000 km of streams [USFS 1993]. Many of the mines generating acid are abandoned, and the costly remediation of these sites often falls under governmental responsibility [Ferderer 1996]. Acidic, heavy metal-rich waters are generated through the oxidation of pyrite (FeS_2), a mineral containing iron and sulfur. The oxidation of pyrite, which produces sulfuric acid and Fe^{2+}, can occur naturally in catchments with pyritic bedrock, such as the upper portion of the Snake River basin near Keystone, Colorado [McKnight et al. 2002], and is referred to as *acid rock drainage*.

AMD streams are characterized by extremely low pH, ranging from negative values found in the Iron Mountain Mine in California to a mildly acidic 5.0 [Nordstrom and Alpers 1999]. At low pH values, many heavy metals that can be toxic in small concentrations are solubilized into stream water. As these waters are diluted, the metals can precipitate, coating sediments and disrupting benthic life and the aquatic food chain [Niyogi et al. 1999]. Thus, both low pH and high heavy metal concentrations stress aquatic ecosystems.

Through the mining process, rocks are removed from the subsurface and crushed to increase the extractability of metals, such as gold, silver, lead and others.

The leftover waste rock, or *tailings*, are piled on the surface, where oxygen and water infiltrate, causing the weathering of pyrite. Sulfide-containing minerals, which typically comprise most of the ore material in mine tailings, are prone to oxidation. Pyrite (isometric FeS_2) is used as the primary model for studying oxidation processes in mine waste because not only is it present in ores containing gold, silver, copper and zinc, but it is also associated with coalfields in the United States and elsewhere in the world [Nordstrom and Southham 1997]. Other sulfide minerals present in waste rock are marcasite (orthorhombic FeS_2), chalocopyrite ($CuFeS_2$), arsenopyrite (As FeS_2) and pyrrhotite (FeS). Exposure of these minerals to water and oxygen oxidizes them, generating sulfuric acid and freeing the other metals associated with the complex. At pH values below 4.5, the presence of iron-oxidizing microorganisms controls the rate of oxidization [Singer and Stumm 1970]. The acidic, heavy metal–laden waters that drain from mine adits and tailings piles is called *Acid Mine Drainage* (AMD).

Iron is the most explored of the biogeochemical cycles in AMD environments. Abiotic iron reactions are important in these environments and are accelerated in the presence of microorganisms, which are discussed later in this chapter. At circumneutral pH, oxygen is the primary oxidant for pyrite, which generates sulfuric acid, free hydrogen ions and either Fe^{3+} or Fe^{2+} (Equation 5).

$$4FeS_2 + 15O_2 + 2H_2O \rightarrow 4Fe^{3+} + 8SO_4^{2-} + 4H^+$$

or

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+$$

Equation 5. Pyrite oxidation under circumneutral pH

Under acidic conditions, iron-oxidizing bacteria catalyze the oxidation of Fe^{2+}, generating Fe^{3+}, which further oxidizes more pyrite (Equation 6).

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \quad \textit{bacterial}$$
$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad \textit{chemical}$$

Equation 6. Bacterially mediated pyrite oxidation

Because of the pyrite oxidation, the iron species chemically combines with other substances in the water to form a variety of iron oxyhydroxides. The waters that carry these complexes often mix with circumneutral pH waters, either from other tributaries, groundwater or hyporheic discharge, and increase the overall stream pH. This, in turn, causes many of these complexes to precipitate out of solution and coat the bottom of a streambed, often as a red-colored coating. This coating, which is a very strong indicator for AMD-impacted streams, can make sediment-bound nutrients and habitats less available for macroinvertebrates or microbial life [Niyogi et al. 1999].

Sunlight is a significant factor in iron cycling in many streams because iron is photoreactive, which may be essential to consider in acid streams because of the abundance of iron. Iron photochemistry in these watersheds may only be important during the late spring, summer and early fall months, because, for the majority of the year, many of these streams are snow-covered. Photoreduction of iron may be important to the fate of nitrite in AMD systems, particularly during the daytime hours when hydrous iron oxides and dissolved Fe^{3+} are reduced by sunlight [McKnight et al. 2001]. The abiotic nitrite reactions may also be important in the cycling of organic

matter in these low pH streams. NO_2^- can be a strong competing scavenger of hydroxyl radicals compared with organic compounds [Zhang and Bartlett 2000]. Dissolved organic carbon (DOC) concentration and composition are also affected in streams where iron oxyhydroxides are present, such as in streams impacted by acid rock or by AMD [McKnight et al. 2002]. The interaction of nitrite, DOC and iron compounds, which may be important in AMD streams, may be seasonally influenced by DOC flushing, nitrate pulses in snowmelt and increased nitrite production from denitrification. This interaction needs further investigation.

Besides metals and low pH in acid-impacted streams, dissolved organic carbon has received some attention as well. Overall, AMD streams are usually considered to have low organic carbon inputs, allowing for a strong community of autotrophic microorganisms to flourish. However, in these systems, it is important to examine carbon cycling that may sustain heterotrophic life. Marchand and Silverstein [2002] reported that autotrophic iron oxidation was enhanced by the presence of a heterotrophic co-culture (*Acidiphilium acidophilus*). Other investigators proposed that organic acids (e.g., pyruvate) produced by *Acidithiobacillus ferrooxidans* and other iron-oxidizing autotrophs are taken up by acidophilic heterotrophic bacteria [Knief et al. 2003]. Organic ligands produced by algal mats and other microbial life can complex with ferric iron in mine waters [Herbert 2000], which may sustain a heterotrophic community living in the sediment. A stream scale carbon transport experiment was conducted on the naturally acidic Upper Snake River, near Keystone, Colorado. Results from this study showed that fulvic acid was irreversibly sorbed to iron

oxyhydroxides on the streambed [McKnight et al. 2002], which may provide a carbon source to the microbial community living in the sediments of acid-impacted rivers. Even though the fulvic acid fraction may not be the preferred type of carbon for heterotrophic microbes, it is a carbon source, and organisms living in the sediments could adapt to using this type of carbon. In a study on Deer Creek, a pristine stream adjacent to the Upper Snake River, flushing of carbon during snowmelt was prevalent [Boyer et al. 2000]. Because these two watersheds are so close, DOC may be flushed during snowmelt from the soil into the Snake River, as well. As the carbon is flushed through this system, it can sorb to iron or other metal complexes in the streambed, which may provide a source of carbon throughout the year. Thus, even though DOC concentrations in AMD streams may be low, a constant supply of carbon, sorbed to the sediments, to sustain heterotrophic life may exist.

Many streams impacted by AMD are located in mountain regions where spring snowmelt dominates the hydrograph. During snowmelt, streams affected by AMD often experience high metal concentrations and low pH from the flushing of mine tailings and adits. In a study examining acid-impacted streams in Summit County, Colorado, the annual maxima concentrations of zinc occurred during snowmelt in three AMD-impacted streams [Brooks et al. 2001]. A naturally acidic stream was also included in this study and showed that, because of the dissemination of pyrite throughout the catchment, stream concentrations of zinc did not peak during snowmelt.

Acid Mine Drainage: An Extreme Environment for Life

Many microorganisms cannot function in acidic environments, because the optimum pH for many organisms in natural environments is from 5 to 9 [Madigan 2000]. High concentrations of H^+ ions can cause the cell wall to rupture because of osmotic pressure, thus killing the organism. Some organisms have adapted their cell walls to tolerate, or even prefer, acidic environments. For obligate acidophilic bacteria, an increase in pH can actually be detrimental. At a neutral pH, the cytoplasmic membrane of these bacteria dissolves, and the cells lyse. In these organisms, the high concentration of hydrogen ions is required for membrane stability [Johnson 1998, Schleper et al. 1995].

The total concentration of dissolved solids in AMD environments is often high. Concentrations of iron of 1 to 30 g/L and sulfate from 3 to as high as 100 g/L have been reported (see [USFS 1993] for overview). These high concentrations can cause osmotic pressure stress on organisms, because the waters are often *hypertonic*—they have higher concentrations of dissolved constituents outside as compared to within the cytoplasm. A cell in a hypertonic environment tends to lose water, which causes it to shrink and dehydrate. The primary function of the cell wall is to regulate osmotic flow. Organisms living in AMD environments have often adapted their cell walls to control the amount of water retained in the cell, so they do not dehydrate. Halophilic bacteria have other mechanisms for maintaining osmotic pressure, including synthesis of intracellular electrolytes [Fortin et al. 1994].

Another aspect of AMD environments that makes it difficult for life is the concentration of heavy metals. Metals commonly mobilized in these environments are Ag, Au, Co, Cu, Pb, As, Cd, Pb, Al, Fe, Mo and Zn [Nordstrom and Alpers 1999]. Though many trace metals are necessary for the formation of enzymes [Madigan 2000], high concentrations of metals can interfere with enzyme function by competing with or replacing another particular metal co-factor, resulting in metal toxicity, which, in turn, results in a slowed or complete cessation of cellular growth.

Some microorganisms have developed elaborate biochemical mechanisms to reduce the effect of metal toxicity. Microbes have been shown to bind metals to the cell wall, transform metals to nontoxic ionic states or precipitate metals intracellularly (see [Chapelle 2001] for review). This acclimation to metals can be accomplished by inducing appropriate enzymatic systems or repressing other enzymes. These changes occur at a genetic level but do not change the function of the microbe's genome. Other microbes have initiated point mutations or deletions of genetic material to accomplish tolerance or resistance to high metal concentrations. The acclimation to metals is evidenced by a characteristic lag time in the growth curve that follows exposure to high concentrations of various metals [Beveridge 1981].

Microbial Diversity in AMD

Extreme conditions in high mountain streams—temperature variation, a hydrograph characterized by seasonal high and low flows, low carbon flux and added chemical stresses from AMD—constitute stresses to bacterial life. Little is known about

the population dynamics of microorganisms in the environment, and understanding the microbial community structure should provide important information on the cycling of nitrogen and other biogeochemically active elements in AMD watersheds. Researchers have developed special enrichment techniques for characterizing natural microbial populations in soils, sediments and streams [Schmidt 1992] (and see [Caldwell et al. 2002] for review). However, environmental conditions that play a significant role in the growth of bacteria in AMD-impacted watersheds are very difficult to replicate in typical laboratory settings [Johnson 1995]. To complicate the situation, it is estimated that far less than 1% of organisms in the environment can be cultured, rendering traditional techniques for monitoring microbial communities ineffective [Pace 1996]. Because environmental microbes are difficult to culture, it is important to utilize other approaches to assess the microbial diversity in natural environments. Analysis of bacteria membrane phospholipids and fatty acids (PLFA) has been used to characterize microbial populations in the environment [White et al. 2002]. A benefit of PLFA is that it can provide information about population activity because phospholipids tend to disappear very quickly from nonviable cells. In addition, PLFA can provide quantitative information about populations, or at least about relative population sizes. Other researchers have used analysis of enzyme activity to characterize microbial activities without inferring species identification (see [Tabatabai 1994] for review).

In the 1990s, advances in molecular technology enabled the characterization of an environmental microbial community by isolating and sequencing genomic material

(Figure 2-2). Universal primers have been developed for isolating genomic fragments from archaeal, bacterial and eukaryotic members of a community [Amann et al. 1995]. With the discovery of the polymerase chain reaction (PCR), the method of sequencing the 16S rRNA molecule of each microbial member of a sample became more efficient and far less time consuming. This technology is a very powerful tool for providing insight into the phylogeny of a microbial community. As new microbes are identified with 16S rRNA techniques, our understanding of the tree of life continues to evolve, as does our understanding of the function of many of the organisms that are genetically related based on phylogenetic trees.

These techniques are particularly important when studying the microbial community in extreme environments, where it is often difficult to culture microorganisms. Most of the research on microbial diversity in AMD environments has focused on acid generation and the species found in mine tailings; there has been little research on the effect of AMD on the microbial community downstream. Culturing acidophilic bacteria can be difficult [Johnson 1995]; thus, using molecular techniques is an important tool necessary for describing the microbial community in these AMD-impacted environments.

Various studies used molecular techniques to examine the abundance of the iron-oxidizing bacterium *Acidithiobacillus ferrooxidans* (formerly *Thiobacillus ferrooxidans*) and the importance of this species in AMD environments [Bond et al. 2000, Edwards et al. 1999, Schrenk 1998]. Several other studies indicated that *Leptospirillum ferrooxidans* is also important in the oxidation of the iron-containing pyrite mineral in

some AMD environments, particularly at elevated temperature (~38°C), and Banfield and co-workers studied the influence of an Archaea capable of iron oxidization in AMD environments [Bond et al. 2000, Edwards et al. 1999, Schrenk 1998]

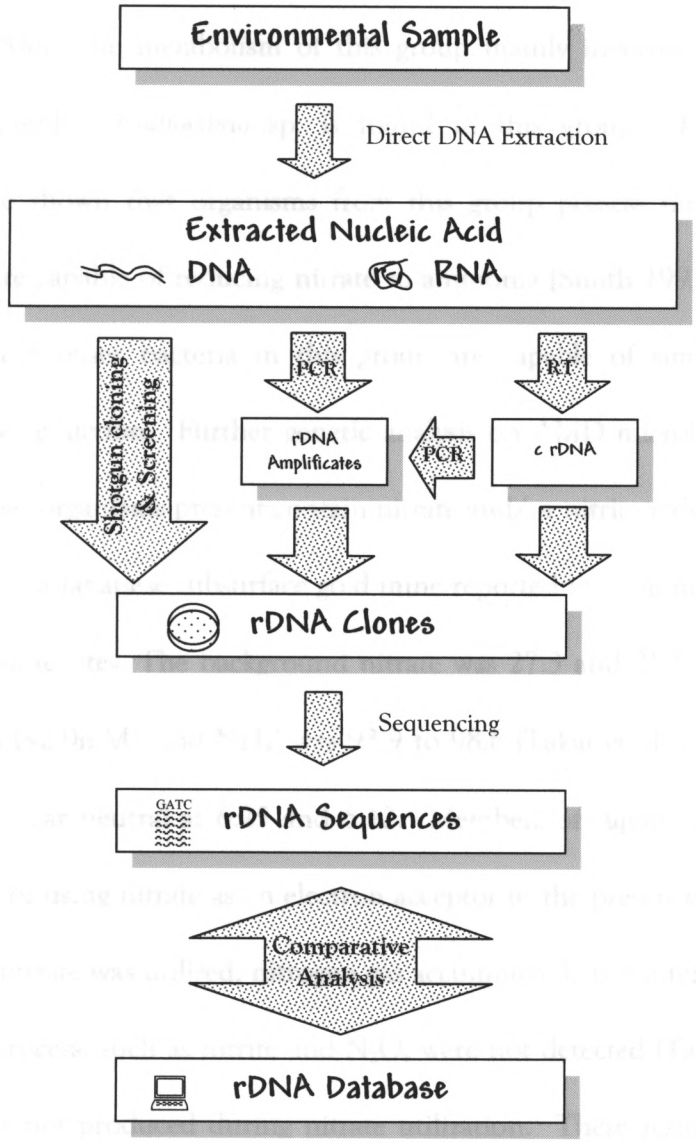

Figure 2-2. Flowchart for characterizing the microbial community by 16S rRNA Adapted from M. Bauer [Amann et al. 1995]

Potential for Denitrifiers in AMD Environments

In addition to their studies of acidophilic microbial communities involved in iron and sulfur cycling at the Iron Mountain mine site discussed above, researchers have found a number of delta proteobacteria that have not previously been described [Bond et al. 2000]. The metabolism of this group mainly involves iron and sulfur cycling; for example, *Desulfovibrio* sp. is found in this group. However, several researchers have shown that organisms from this group possess nitrate and nitrite reductase and are capable of reducing nitrate to ammonia [Smith 1992, Telang 1997]. It is possible that other bacteria in this group are capable of similar metabolism involving nitrate reduction. Further genetic analysis on AMD microbes is needed to determine if other organisms present contain nitrate and/or nitrite reductases.

A study of a Japanese subsurface gold mine reported measurements of nitrogen species in two mine sites. The background nitrate was 27.3 and 25.5 μM, nitrite was below detection (<2.0mM), and NH_4^+ was 93.9 to 98.6 [Takai et al. 2002]. pH of the mine water was near neutral at 6.25 and 6.12. Members of *Aquificales* were isolated that are capable of using nitrate as an electron acceptor in the presence of hydrogen or thiosulfate. As nitrate was utilized, nitrogen gas accumulated, and intermediates in the denitrification process, such as nitrite and N_2O, were not detected [Takai et al. 2002]. Ammonium was not produced during nitrate utilization. These results indicate that denitrification may occur in this subsurface gold mine.

Other sources of nitrogen in these systems could also result from ammonium-nitrate fuel oil explosives used in blasting, cyanide degradation from heap leaching

operations, and even fertilizers used in mine reclamation. For example, water from a mine in Montana had more than 400 mg/L of nitrate [USEPA/USDOE 1996].

The research presented in this dissertation examines nitrate reduction and, more specifically, denitrification in AMD-impacted streams. With increasing nitrogen deposition rates [Williams 2000], it is likely that nitrate will enter AMD-impacted mountain streams through snowmelt or direct precipitation, but the fate of nitrate in these environments is not known. Because of the many health and ecosystem risks associated with excess nitrate, it is important to determine what happens to nitrate in AMD waters. In addition, adaptation to more recent inputs of AMD products (iron, sulfur, protons and heavy metals), along with nitrate, may have caused a shift in the microbial populations in these environments. The identification of AMD products may be important in predicting the capacity of microbial populations to change biogeochemical element cycling, in turn changing the export of contaminants to soil and water downstream.

CHAPTER 3: SITE DESCRIPTIONS AND METHODS

Site Descriptions

Abandoned mines dot the landscape of the Rocky Mountains in Colorado. Initial studies of denitrification potential were conducted in laboratory microcosms, using inocula consisting of sediment and surface water from five AMD-impacted streams. Later studies of the effect of environmental conditions and population composition used samples from two AMD-impacted streams to examine the microbial community capable of denitrification that exists in AMD streams. In addition, a naturally acidic mountain stream was examined to give a comparison between processes in an AMD-impacted stream and in a stream that has a naturally low pH with high heavy metal concentrations. A pristine stream in the same geographic region served as a comparison of microbial processing of nitrogen in a typical mountain stream. Other researchers have also studied the streams selected [Bird 2003, Boyer et al. 2000, Brooks et al. 2001, McKnight and Bencala 1990, McKnight et al. 2002], providing some background data on the sites. All the streams used for the experiments on the influence of water quality and seasonal conditions are located in Summit County, Colorado, just west of the Continental Divide, as shown in Figure 3-1.

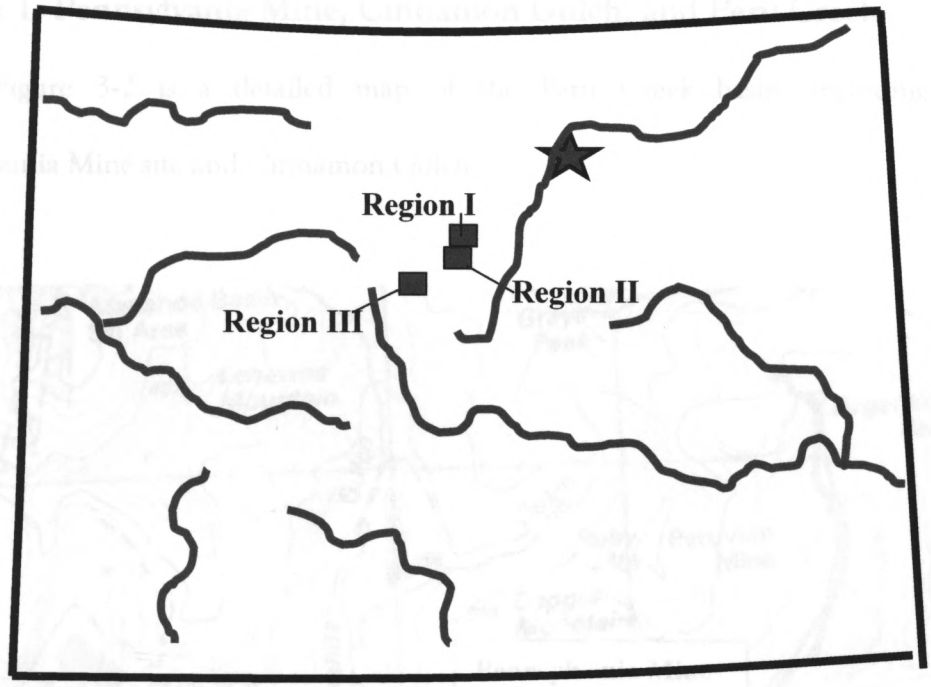

Figure 3-1. Colorado map with sampling site regions. ★ represents Denver

Region I comprises the Pennsylvania Mine, Cinnamon Gulch and Peru Creek, as described below. Snake River, a naturally acidic mountain stream, was located in Region II, near Keystone, Colorado, along with Deer Creek, a pristine mountain stream. A stream draining St. Kevin Gulch and a nearby spring from a large tailings pile were in Region III, located near Leadville, Colorado. Microcosm studies with sediment and surface water collected from Regions I, II and III are reported in Chapter 4. The second portion of the research focused on the interactions between water chemistry, nitrogen species, denitrification potential and microbial diversity in two regions: drainage from the abandoned Pennsylvania Mine and Peru Creek (Region I) and the naturally acidic Snake River and the pristine Deer Creek (Region II) during snow cover, snowmelt and summer flow (Chapters 5 and 6).

Region 1: Pennsylvania Mine, Cinnamon Gulch, and Peru Creek

Figure 3-2 is a detailed map of the Peru Creek basin, including the Pennsylvania Mine site and Cinnamon Gulch.

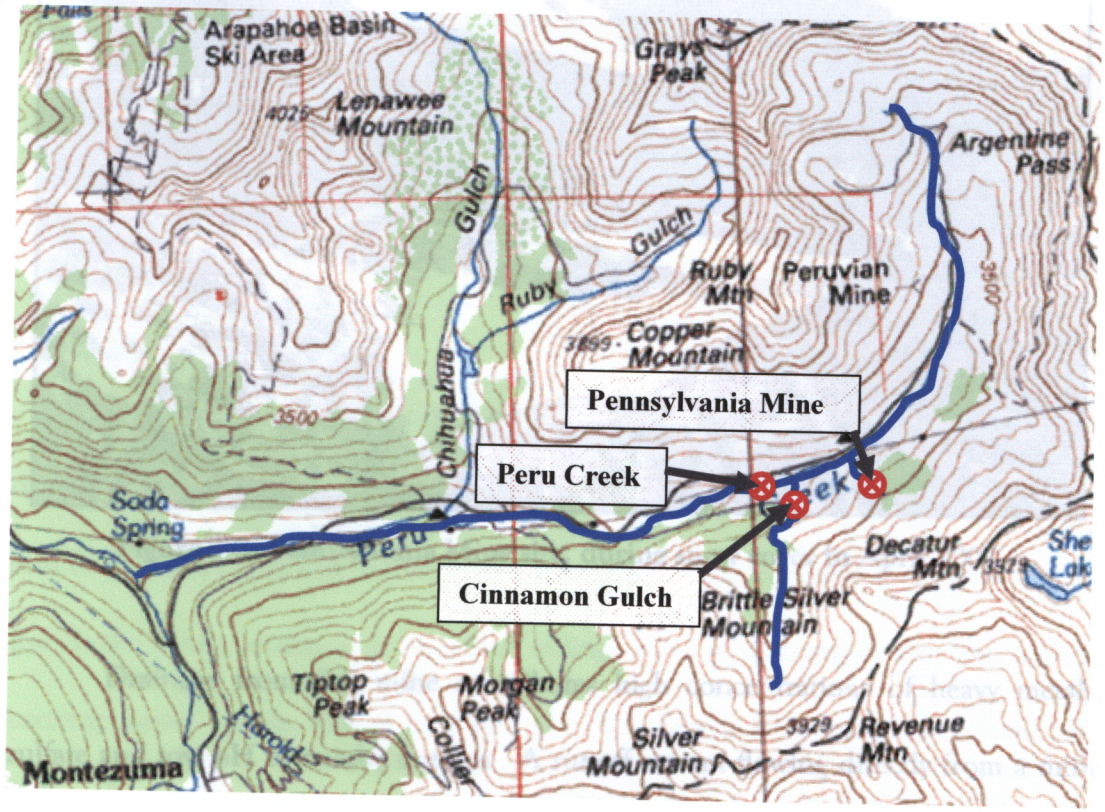

Figure 3-2. Region I: Pennsylvania Mine, Cinnamon Gulch and Peru Creek

Pennsylvania Mine

The Pennsylvania Mine (Figure 3-3) is an abandoned gold, silver, lead, zinc and copper mine that operated from 1885 to 1953 near Keystone, Colorado, in the headwaters of the Colorado River. The mine site is located in an alpine/subalpine environment, between 10,950 and 11,500 feet elevation.

Figure 3-3. Pennsylvania Mine site, during snow cover in April 2003

Drainage from this mine contributes high concentrations of heavy metals, sulfate and low pH to nearby streams. A runoff stream flowing directly from a mine adit was selected for examination of the direct effect of mine runoff on water chemistry and nitrogen cycling of AMD-impacted streams.

Cinnamon Gulch

Cinnamon Gulch (Figure 3-4) is a small stream near Pennsylvania Mine that drains from the Silver Spoon Mine near its source, down through Delaware Mine, and into Peru Creek [Bird 2003]. This site was used to assess downstream effects of AMD after collection into a stream.

Figure 3-4. Cinnamon Gulch converging into Peru Creek during snowmelt in June 2003

Figure 3-5. Sampling Peru Creek during snowmelt in June 2003

Peru Creek

Peru Creek (Figure 3-5), a second-order stream that collects much of the drainage from several mines in the region near Keystone, Colorado, has a watershed area of 41.4 km^2. The headwaters of this stream are pristine; however, along its path to the Snake River, drainage from many tailings piles is discharged into the stream, carrying heavy metals, sulfate and low pH. The Snake River eventually flows into Dillon Reservoir, a major drinking water source for the Denver metropolitan area. Peru Creek, a higher-order stream than the runoff stream emanating directly from Pennsylvania Mine and Cinnamon Gulch, was chosen to examine the effects of dilution of AMD on water chemistry and microbial stream processes.

Region II: Snake River and Deer Creek Comparison Sites

The Upper Snake River and Deer Creek are located near the Peru Creek Basin, just south of Montezuma, Colorado (see Figure 3-6).

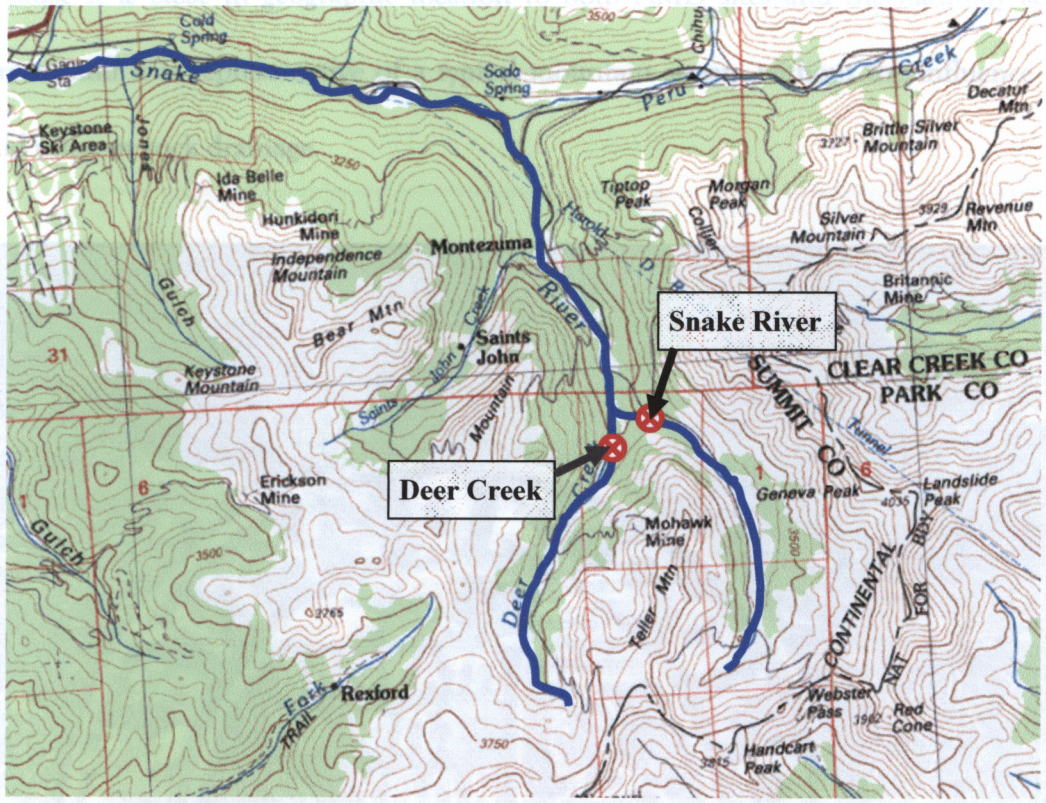

Figure 3-6. Region II: Map of Snake River and Deer Creek

Snake River

Two nearby sites were chosen to compare the effects of AMD in other mountain streams in the area. The Upper Snake River (Figure 3-7) is a second-order naturally acidic stream influenced by the pyritic rocks in the drainage areas [McKnight et al. 2002]. The watershed area draining into the sampling site is approximately 11.8 km^2. This stream was used as a naturally acidic control to compare with AMD-contaminated streams.

Deer Creek

Deer Creek (Figure 3-7) is a pristine mountain stream, draining about 10.5 km^2, that flows into the Upper Snake [McKnight et al. 2002]. This stream was selected

because it is close in geographic location to both AMD sites and the naturally acid stream and it is representative of a typical mountain stream without the influence of acid mine or rock drainage.

Figure 3-7. Convergence of pristine Deer Creek (top) and naturally acidic Snake River (bottom) in August 2002

Region III: St. Kevin Gulch, Griffin Mine

St. Kevin Gulch (Figure 3-8), a small tributary to Tennessee Creek that drains approximately 10 km^2 [Niyogi et al. 1999], is in the headwaters of the Upper Arkansas River near Leadville, Colorado, and has a pH of around 3.0 [Broshears et al. 1996]. The stream flows past the base of a large tailings pile left from the abandoned Griffin

Mine [Jessen 1999] (Figure 3-9). A small spring, with a pH around 2.6, flowing from the tailings pile (Figure 3-10) was also sampled for this study.

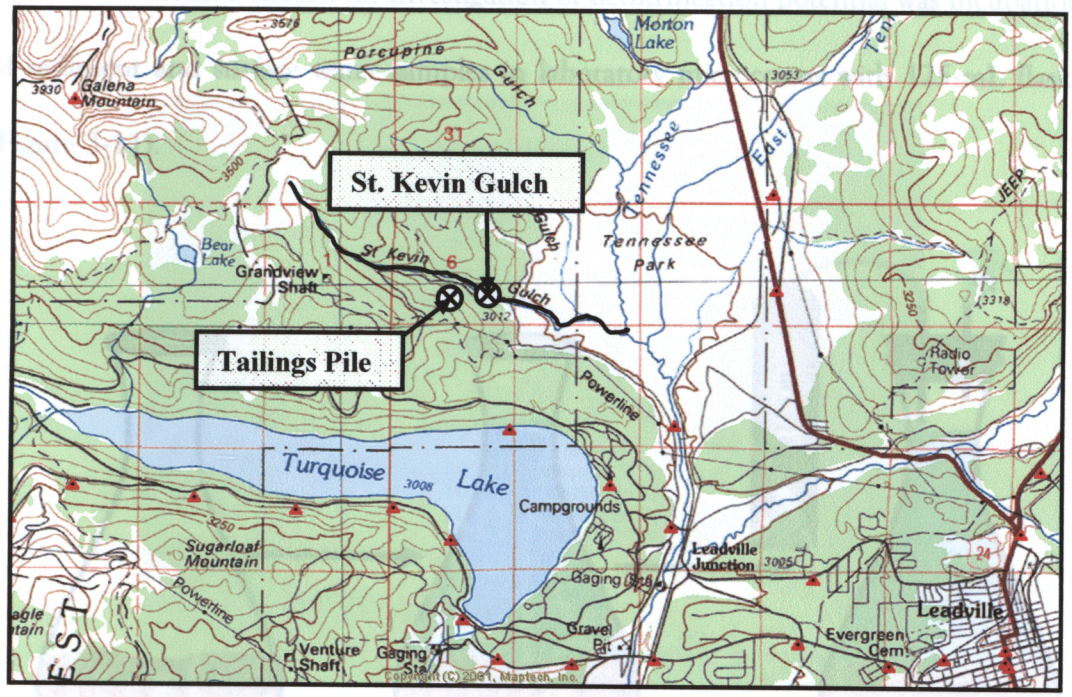

Figure 3-8. Region III: Map of St. Kevin Gulch and tailings pile spring

Figure 3-9. St. Kevin Gulch flowing at the base of tailings from the Griffin Mine in July 2002

Figure 3-10. Spring flowing from Griffin Mine tailings in July 2002

Methods

Denitrification Potential Assays

The method chosen for investigation of denitrification potential was incubation of sediment and surface water samples in laboratory microcosms with 125-mL flasks (Figure 3-11).

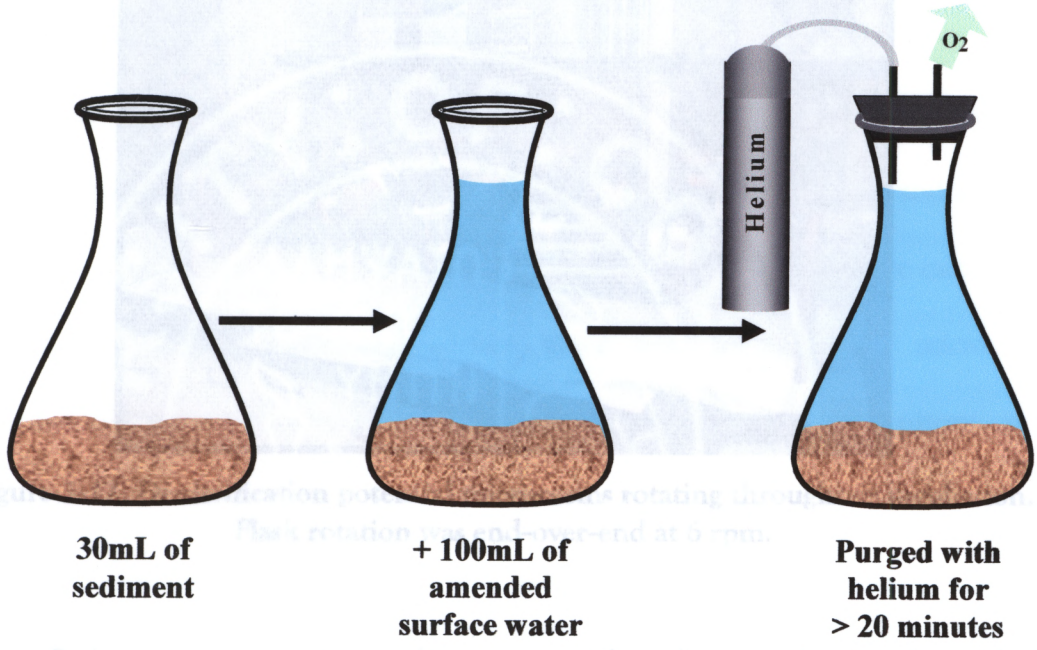

Figure 3-11. Denitrification potential microcosm setup

Sediment was collected in mason jars from three locations at each site and mixed to ensure homogeneity. Sediment was stored at 4°C for a maximum of 24 hours until analysis. Triplicate microcosms from each site were set up as follows: 30mL of mixed saturated sediment was added to a 125mL flask, followed by 100mL of surface water amended with 5mg/L NO_3-N (as KNO_3). Flasks were stoppered and purged with UPH helium gas for >20 minutes to remove headspace oxygen and were rotated end to

end (6 rpm) at room temperature for the duration of the experiment (Figure 3-12). Water and gas samples were collected at several times during the incubations, with helium gas added to replace the volume removed (5mL headspace and 10mL water).

Figure 3-12. Denitrification potential microcosms rotating throughout incubation. Flask rotation was end-over-end at 6 rpm.

Preliminary microcosm studies were conducted using the acetylene block method for denitrification potential analysis, with sediments collected from Gamble Gulch, a stream receiving AMD from an abandoned mine in Boulder County, Colorado (Appendix I). However, this method was subsequently abandoned because of interferences between heavy metals, sulfide and acetylene (see [Knowles 1982] for review), which could decrease the amount of acetylene present to block the production of nitrogen gas, resulting in an underestimation of denitrification potential.

Experiments were conducted using surface water alone, surface water with biologically active sediments and gamma-irradiated sterile surface water and sediments to assess if biological activity was present in mainly the sediments or in the water column as well. Gamma-irradiation was selected to produce killed controls, because autoclaving these sediments changed the oxidation states of the metals present in the sediments and surface water. Mass balance recoveries were conducted on microcosms after 0 and 336 hours (14 days).

For complete denitrification, samples were collected for determination of nitrate, nitrite, nitrogen gas, ammonium, dissolved metal concentrations and pH was measured at each time point. Nitrogen mass balances for each flask were calculated. Cinnamon Gulch sediment and surface water were used for the microcosm experiments to assess factors affecting denitrification augmented with nitrate, pH, DOC and iron species. Water quality characteristics were monitored over the course of time. Controls for each experiment were microcosms with Cinnamon Gulch sediment and surface water at ambient conditions with only nitrate added. Experiments to assess effects of pH were conducted by adjusting the ambient pH (4.14) of microcosms to 2.6, 3.5, 4.25 or 6.0. Microcosm studies to assess the influence of added electron donors were conducted by adding 5mM glucose or acetate (as sodium acetate) as carbon to two sets of microcosms. In addition, natural organic matter was obtained by leaching leaves from nearby willow trees (collected in late August 2002) in nanopure water (US Filter) for 24 hours and the eluent added as 5mM DOC to another set of microcosms. Hydrogen gas (10% headspace concentration) was added to

another set of microcosms to assess the potential for hydrogen to serve as an electron donor for denitrification. Additional experiments were conducted to assess the effects of added iron; concentrations of iron were added to the surface water at 50 and 200 mg/L Fe^{2+} (as $FeSO_4 \cdot 7H_2O$) and 50 and 200 mg/L Fe^{3+} (as $FeCl_3 \cdot 6H_2O$) for several sets of microcosms. Ambient Cinnamon Gulch sediment and surface water were used for controls for these tests.

Water Chemistry Analyses

Microcosm Studies (Chapter 4)

Nitrate samples were filtered through 0.1μm Millipore filters, preserved by freezing, and analyzed using a Dionex 500 Ion Chromatograph with carbonate buffered eluent (1.8 mM Na_2CO_3/ 1.7 mM $NaHCO_3$, pH 7.5) at a flow rate of 2.00 mL/minute through an IonPac AS4A analytical column and an AG4A guard column. Ammonium samples were filtered through 0.1 μm Millipore filters, preserved with concentrated sulfuric acid to a final pH of 2, and analyzed using a Dionex 300 Ion Chromatograph with a gradient method using eluent ranging from 2.5 to 25 mN sulfuric acid over the course of the run at 1.5 mL/minute through a CS12A analytical column and CG12A guard column. Nitrite samples were filtered through 0.1μm Millipore filters, and frozen until analysis. Nitrite was reduced to nitric oxide with 0.5mL of 2% sodium iodide added to 4mL of glacial acetic acid, exposed to ozone, and passed through a Sievers NOA 280 chemiluminescent nitric oxide detector. Nitrogen gas was analyzed using a Hewlett-Packard 5890 gas chromatograph equipped with a micro-thermister detector (TCD) and a 30′ metal column with polymer packing for 30°C temperature

separation of N_2, O_2 and CO. This device consists of two zero dead volume (four- and six-port) valves (Valco-Inc.) and a pressure gauge (0–30 PSI) connected in-line between the carrier gas source (UHP Helium, General Air) and the GC column. DOC samples were filtered through ashed GF/F filters and analyzed using a Shimadzu TOC-5050 Total Organic Carbon Analyzer. For heavy metal analysis, microcosm water samples were filtered through 0.1 μm Millipore filters and preserved with trace metal grade hydrochloric acid (2% v/v) and analyzed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (Leeman Labs, DRE (direct reading echelle)). Iron species (Fe_{Total} and Fe^{2+}) were measured using the FerroZine method described by To et al. [To et al. 1999]. Fe^{3+} concentration was determined by subtracting the Fe^{2+} concentration from the total iron concentration.

First-order specific denitrification potential rate constants were calculated using the average of nitrate concentration data from the three replicate flasks, collected at 0, 24, 48 and 72 hours of incubation by linear regression to the model

$$\ln\left[\frac{N}{N_o}\right] = -k * t ,$$

where N is the average nitrate-nitrogen concentration (μmole/L), N_o is the average initial nitrate-nitrogen concentration (μmole/L), t is elapsed time (in days) and k is the specific first-order reaction constant (μmole-NO_3-N g-sediment^{-1} day^{-1}). The regression slope was divided by the average sediment mass per flask (dry weight) to calculate the specific first-order denitrification rate constant, k.

Seasonal Water Chemistry and Denitrification Potential Analysis (Chapter 5)

Stream water samples and snow, when present, were collected from each site and taken to the lab for immediate analysis and preservation. Snow samples were melted at 4°C and preserved for analysis as surface water samples. pH was measured with an Orion pH probe. Nitrate samples were filtered through 0.1 μm Millipore filters and preserved by freezing and analyzed using a Dionex 500 Ion Chromatography with carbonate- buffered eluent (1.8 mM Na_2CO_3/ 1.7 mM $NaHCO_3$, pH 7.5) at a flow rate of 2.00 mL/min through an IonPac AS4A analytical column and an AG4A guard column and a conductivity detector. Ammonium samples were filtered through 0.1 μm Millipore filters and preserved with 0.2 mL concentrated sulfuric acid (per 60 mL, final pH of 2) and analyzed using a Dionex 300 ion chromatograph with a gradient method using eluent ranging from 2.5 to 25 mN sulfuric acid over the course of the run at 1.5 mL/min through a CS12A analytical column and CG12A guard column and a conductivity detector. Nitrite samples were filtered through 0.1 μm Millipore filters and frozen until analysis. Nitrite was reduced to nitric oxide with 0.5 mL of 2% sodium iodide added to 4 mL of glacial acetic acid and passed through a Sievers NOA 280 chemiluminescent nitric oxide detector. DOC samples were filtered through ashed GF/F filters and analyzed using a Shimadzu TOC-5050 Total Organic Carbon Analyzer. For metal analysis, microcosm water samples were filtered through 0.1 μm Millipore filters and preserved with trace metal-grade hydrochloric acid (2% v/v) and analyzed by the University of Colorado Geology Department lab for trace metals by Inductively Coupled Plasma- Mass Spectrometry (ICP-MS).

First-order specific denitrification potential rate constants were calculated as described above.

Microbial Diversity Community Analysis

Given the extreme environmental conditions in AMD streams located in high mountain watersheds and the possibility of novel organisms that would be assessed by phylogenetic characteristics, it was decided to investigate the diversity of the sediment microbial communities using 16S rRNA analysis. Sediment samples for microbial diversity were collected in triplicate sterile tubes from Pennsylvania Mine, Peru Creek, Snake River and Deer Creek during snow cover (April 22, 2003), snowmelt (June 6, 2003), and summer (July 21, 2003) flow conditions.

Sediment Sample Collection

Triplicate sediment samples were collected during snow cover (April 22, 2003), snowmelt (June 6, 2003) and summer (July 21, 2003) from each of the acid-impacted mountain streams, as well as from the pristine site, into 15 mL sterile tubes and frozen until analysis.

Surface Water Sample Collection and Analysis

Surface water samples were also collected and taken to the laboratory for immediate preservation and water chemistry analysis to characterize the environmental provenance of the sites. pH was measured with an Orion pH probe. Samples for anion determination were filtered through 0.1 μm Millipore filter and preserved by freezing

and analyzed using a Dionex 500 Ion Chromatograph with carbonate- buffered eluent (1.8 mM Na_2CO_3/ 1.7 mM $NaHCO_3$, pH 7.5) at a flow rate of 2.00 mL/min through an IonPac AS4A analytical column and an AG4A guard column and a conductivity detector. Samples for ammonium determination were filtered through a 0.1 μm Millipore filter and preserved with concentrated sulfuric acid to a final pH of 2 and analyzed using a Dionex 300 Ion Chromatograph with a gradient elution of 2.5 to 25 mN sulfuric acid at 1.5 mL/min through a CS12A analytical column and CG12A guard column and a conductivity detector. DOC samples were filtered through ashed GF/F filters and stored in ashed amber bottles at 4°C. Samples were analyzed using a Shimadzu TOC-5050 Total Organic Carbon Analyzer, within three weeks of sample collection. For dissolved trace metals, stream water was filtered through 0.1 μm Millipore filters, preserved with trace metal-grade hydrochloric acid, and analyzed by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) (Liberty Series 2, Varian Corporation)

Environmental Genomic DNA Extraction

Total environmental community genomic DNA was extracted using a solvent and bead-beating extraction protocol, modified slightly from the method described by Dojka et al [Dojka et al. 1998], from each triplicate sediment sample and pooled to reduce spatial variation in the stream sediment environment. Briefly, DNA was extracted from ~0.5 g of sediment with 0.7 ml acid extraction buffer (400 mM TRIS pH 8.0, 100 mM EDTA, 6% (wt/vol) SDS), 0.5 ml buffer saturated (pH 7.5)

phenol/chloroform/isoamyl-alcohol (24:24:1) and 0.5 g of 0.1 mm zircon/silica beads (BioSpec Products Incorporated). All procedures were performed in a UV-sterilized AirClean 600 PCR workstation (AirClaen Systems).

PCR Amplification and rDNA Clone Libraries

Community small subunit (SSU) ribosomal RNA (rRNA) genes (rDNA) were amplified by polymerase chain reaction (PCR) using extracted genomic DNA as template and universal primers (below). Each 20 µl PCR reaction contained ~50 ng of extracted DNA template, 1X HotMaster Buffer (Eppendorf), 200µg/ml Bovine Serum Albumin (BSA), 50 µM (each) deoxynucleotide triphosphates, 0.2 µM each forward and reverse primer and 1.25 U of HotMaster Taq DNA Polymerase (Eppendorf). PCR reaction mixtures were incubated in a MasterCycler programmable thermal cycler (Eppendorf) at 94°C for 2 minutes (for initial denaturation), followed by 30 cycles of 94°C for 20 seconds, 52°C for 20 seconds, and 65°C for 90 seconds, followed by a final extension period of 10 minutes at 65°C. The primers used in this study were 515F (universal) (5′-GTGCCAGCMGCCGCGGTAA-3′), and 1391R (universal) (5′-GACGGGCGGTGWGTRCA-3′).

Environmental rDNA clone libraries (collection of randomly selected clones) were constructed from PCR-amplified products purified by gel electrophoresis using the Montage DNA Gel Extraction Kit (Millipore) and cloned using the TOPO TA Cloning Kit (Invitrogen), as specified by the manufacturers. Plasmid DNAs containing inserts were screened by restriction fragment length polymorphism (RFLP) analysis

[Dojka et al. 1998] using Msp I and HinP1 I restriction endonucleases (New England Biolabs). PCR products from rDNA clones with unique RFLP patterns were prepared for DNA sequencing using the ExoSAP-it For PCR Cleanup Kit (USB) and sequenced with twofold coverage (vector primers T3 and T7) using a MegaBACE 1000 DNA sequencer (Amersham Biosciences) and DYEnamic ET Terminator Cycle Sequencing Kit reaction mixtures (Amersham Biosciences).

In total, we constructed 12 libraries, each with either 96 or 192 (see below) randomly selected clones, from individual PCR reactions performed with the universal primer pair 515F and 1391R on each DNA sample. PCR reactions were performed for each replicate DNA and cloned separately to yield 12 universal libraries. PCR reactions of 5 of the 12 DNA samples (Pennsylvania Mine snow cover, Deer Creek summer and all Snake River samples) showed two distinct bands during gel purification. When present, these bands were purified and analyzed separately. No strong differences were noted between the bands; thus, the sequences from the two bands were combined and treated at one library in all further analyses.

Phylogenetic Analysis

DNA sequence analysis, assembly, initial characterization and databasing were performed with the XplorSeq software package [Frank 2003–2004]. Within XplorSeq, raw DNA sequencer trace data was analyzed with the PHRED software package [Ewing and Green 1998, Ewing et al. 1998] to assign bases and quality scores. Partial sequences were assembled into contiguous sequences using the PHRAP software package [Ewing

and Green 1998, Ewing et al. 1998]. rDNA sequences were initially compared with a current database of genetic sequences (GenBank) using the BLAST (Basic Local Alignment Search Tool) network service [Altschul et al. 1997] to determine their approximate phylogenetic affiliation. Complete rDNA sequences were exported from XplorSeq and aligned to other known SSU rRNA sequences using the ARB software package [Strunk et al. 1998]. Sequence alignments used to infer phylogenetic relationships were created using the Lane mask [Lane et al. 1985], which excludes hypervariable regions of the SSU-rRNA sequence. Sequence information from the sites with two bands showed little difference and, thus, were pooled together by site and sampling time.

CHAPTER 4:

Denitrification in Stream Sediments Impacted by Acid Mine Drainage: Effects of pH, Various Electron Donors and Iron

Abstract

Acid mine drainage contaminates thousands of streams in the western United States. At the same time, nitrogen loading to many mountain watersheds is increasing due to atmospheric deposition of nitrate and increased human use. Relatively little is known about nitrogen cycling in acidic, heavy metal-laden streams; however, it has been reported that one key process, denitrification, is inhibited under low pH conditions. The objective of this research was to investigate the capacity for denitrification in acidified streams. Denitrification potential was assessed in sediments from several Colorado AMD-impacted streams, ranging from pH 2.60 to 4.54, using microcosm incubations with fresh sediment. Added nitrate was immediately reduced to nitrogen gas without any lag period, indicating that denitrification enzymes were expressed and functional in these systems. First-order denitrification potential rate constants varied from 0.059 to 0.323 day^{-1}. The pH of the microcosm water increased between 0.23 to 1.49 pH units during denitrification. Additional microcosm studies were conducted to examine the effects of initial pH, various electron donors and iron (added as Fe^{2+} and Fe^{3+}). The addition of ferric and ferrous iron decreased observed denitrification potential rates. An increase in the initial pH increased the denitrification potential rate; likewise, addition of glucose and natural organic matter

(NOM) also stimulated denitrification potential. The addition of hydrogen had little effect, however, and denitrification potential activity actually decreased after acetate addition. These results suggest that denitrification can occur in AMD streams, and if stimulated by environmental factors, denitrification might reduce acidity.

Introduction

Mountain watersheds are increasingly being impacted by multiple sources of contamination. For example, acidified streams receiving mine drainage are now subject to nitrate loads from atmospheric deposition that may exceed the nitrogen cycling capacity of the stream sediments, which may lead to nitrogen saturation and increased export of nitrate. The EPA estimates that AMD contaminates 7,000 km of stream in the eastern United States, and the U.S. Forest Service reports that 8,000–16,000 km of streams are affected by AMD in the forest service land of the western United States alone [EPA 1994, U.S. 1993]. AMD, which is caused by the oxidation of pyrite minerals in waste rock and tailings, generates iron and sulfuric acid [Edwards et al. 1999]. Increased acidity causes leaching of heavy metals present in the rocks to nearby streams, damaging aquatic habitat and water quality.

Nitrogen deposition is increasing in the Rocky Mountains of Colorado, resulting in nitrogen saturation and increased amounts of nitrate exported in surface waters [Williams 2000]. Biological denitrification is the primary process for removal of nitrate from water and soil by reduction to nitrogen gas. Most environmental denitrification is carried out by facultative heterotrophic bacteria under anoxic conditions, where nitrate is the electron acceptor for respiration. The optimum pH

range for complete reduction of nitrate to nitrogen gas is considered to be between 6 and 8; below this optimal pH, N_2O is the dominant end product [Knowles 1982, Simek et al. 2002]. There have been reports of biological denitrification in acidic bogs and forest soils; however, in a study examining denitrification in acidic soils, virtually no N_2 was formed below pH 5, suggesting that denitrification was inhibited by low pH [Christensen 1985]. Another acidic soil study demonstrated that nitrate-reducing potentials decreased with decreasing pH, and the proportion of intermediate products, NO and N_2O, produced from nitrate reduction increased [Blosl and Conrad 1992, Nagele and Conrad 1990a, 1990b]. In contrast, denitrifiers have been isolated from soil with a pH tolerance range from 2.9 to 4.8, with an optimum pH of 3.9 [Parkin 1985]. However, denitrification rates of these isolates were lower than denitrifiers found in a nearby neutral pH soil. The inhibition of denitrification in a denitrifying activated sludge culture at low pH has been attributed to the accumulation of nitrite [Glass and Silverstein 1998]. At low pH, nitrite would exist in the protonated form, HNO_2 (pK_a= 3.7), which is thought to be especially toxic to denitrifiers as an uncoupler. Nitrous acid (HNO_2) has also been shown to inhibit denitrification activity at concentrations as low as 0.04 mg/L HNO_2-N [Abeling and Seyfried 1992].

Availability of electron donors may also limit denitrification in mountain streams. Typically, heterotrophic bacteria carry out denitrification, yet organic carbon in AMD streams is limited [Chapelle 2001, Johnson 1998]. Several studies have examined autotrophic denitrification. In a study on material from a nitrate-contaminated aquifer in Canada, acetate, hydrogen gas, elemental sulfur, thiosulfate,

aqueous ferrous iron and pyrite were used as potential electron donors in the denitrification process [Devlin 2000]. All electron donors tested, except for pyrite, stimulated nitrate removal. Fe^{2+} is a potential electron donor for denitrification [Devlin 2000, Schuler 1999, Till 1998], and pyrite minerals producing Fe^{2+} could be a potential source of electrons in AMD environments. Research at the University of Iowa has shown that denitrification by *Paracoccus denitrificans* can be stimulated by hydrogen produced during anaerobic Fe(0) corrosion in water [Till 1998].

AMD typically contains significant concentrations of heavy metal ions, and several studies have examined the effects of metals on denitrification. Iwasaki and Terai found that denitrifiers grown in copper-depleted media produced N_2O as the predominant end product, with little accumulation of N_2 [Iwasaki and Terai 1982]. Slater and Capone showed that Cr, Pb and Mo stimulated total denitrification, whereas Ni depressed the maximum amount of nitrous oxide produced overall in an anoxic salt marsh [Slater and Capone 1984]. Bacterial iron respiration has been reported at AMD sites [Johnson 1998], and ferric iron could interfere with denitrification by acting as an alternate electron acceptor.

Considering this increasing anthropogenic source of nitrogen through atmospheric deposition and the AMD-impacted streams, it is of interest whether acidic heavy metal-laden streams can process the increasing loads of nitrogen, mainly in the form of nitrate. The objectives of this research were to examine several AMD-impacted streams for denitrification potential and to assess the environmental conditions that affect bacterial nitrate reduction at low pH. Denitrification activity from several AMD-

impacted sites was determined by monitoring nitrogen species during microcosm incubations. The effects of pH, electron donors and iron–key factors in AMD catchments–were also examined.

Methods

Site Descriptions

Five acid-impacted sites in the Rocky Mountains of Colorado were chosen for this study. These sites were divided into three regions, as depicted in Figure 4-1. Region I includes two sites, the Pennsylvania Mine and Cinnamon Gulch. The Pennsylvania Mine is an abandoned gold, silver, lead, zinc and copper mine that operated from 1885 to 1953 near Keystone, Colorado, in the headwaters of the Colorado River [Bird 2003]. Drainage from this mine contributes high concentrations of heavy metals, sulfate and acidity to nearby streams and flows directly into Peru Creek, which subsequently flows into Dillon Reservoir, a major drinking water supply for the Denver metropolitan area. A stream flowing directly from one of the mine's adits was used for examination of denitrification in unmixed mine runoff. Cinnamon Gulch is a small stream near Pennsylvania Mine that drains from the Silver Spoon Mine near its source, down through Delaware Mine, and into Peru Creek [Bird 2003]. This site was used to assess downstream effects of acid mine drainage. Region II is a smaller catchment near Region I. The Upper Snake River is a first-order naturally acidic stream influenced by the exposed pyritic rocks in the drainage areas [McKnight et al. 2002]. This stream was used as a comparison between what may be expected to

occur naturally in acidic streams vs. what may be expected in anthropogenic AMD streams. The Region III field site was St. Kevin Gulch (pH 3.0), a small tributary to Tennessee Creek in the headwaters of the Upper Arkansas River, near Leadville, Colorado [Broshears et al. 1996]. The stream flows past the base of a large tailings pile. A small spring flowing from the tailings pile with a pH of around 2.6 was also sampled for this study. Water and sediment samples were collected from each of these five sites during the summer of 2002.

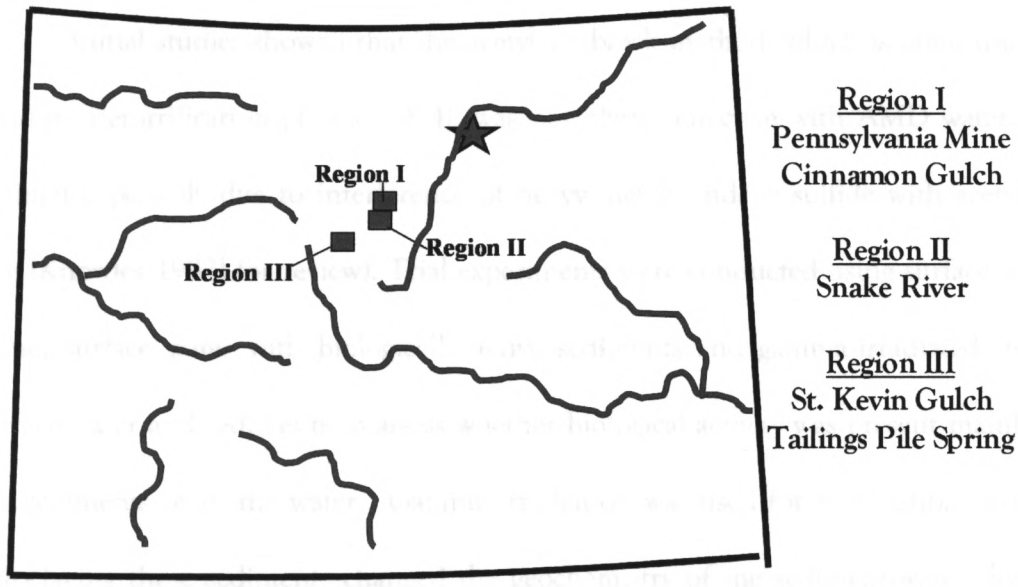

Figure 4-1. Map of sampling regions in the Colorado Rocky Mountains

★ indicates Denver, the capital of Colorado

Denitrification Potential Assays

Sediment from the top 5 cm of the streambed from three locations at each site was collected in Mason jars and mixed to account for variable distribution of microorganisms and to provide an "average" estimate of denitrification potential.

Sediment was stored at 4°C for a maximum of 24 hours until the microcosm incubations were initiated. Triplicate 125 mL flasks from each site were set up as follows: 30mL of mixed sediment was added to each flask with 100 mL of surface water amended with 5mg/L NO_3-N. Flasks were stoppered and purged with helium gas for >20 minutes to attain anoxic conditions and were mixed continuously by end-to-end rotation (6 rpm) at room temperature (22–25°C). Water and gas samples were collected at several time points during the incubations, with helium gas added to replace the volume removed (10 mL water, 5 mL headspace).

Initial studies showed that the acetylene block method, which is often used to measure denitrification [Tiedje 1994], was not always effective with AMD water and sediment, possibly due to interference of heavy metals and/or sulfide with acetylene (see [Knowles 1982] for review). Trial experiments were conducted using surface water alone, surface water with biologically active sediments and gamma-irradiated sterile surface water and sediments to assess whether biological activity was present mainly in the sediments or in the water. Gamma irradiation was used for sterilization because autoclaving these sediments changed the geochemistry of the sediment-water slurries [Wolf and Skipper 1994].

Nitrate, nitrite, nitrogen gas, ammonium, metals and pH were measured at each sample time during all microcosm incubations. In addition, Cinnamon Gulch sediment and surface water were used for microcosm experiments to identify factors affecting denitrification, with nitrate, pH, DOC (for the electron donor experiment) and iron species (for the iron experiment) being monitored throughout the

experiments. Controls were Cinnamon Gulch sediment and surface water at ambient stream conditions. pH was varied by titration with H_2SO_4 or NaOH. Carbon substrate limitation of denitrification was examined by adding 5mM (final concentration) glucose or acetate as carbon to two sets of microcosms. Natural organic matter was prepared by leaching leaves from living willow trees near the sampling sites in nanopure water for 24 hours. The leachate was then added as 5 mM dissolved organic carbon to microcosms. To assess hydrogen utilization as an electron donor in denitrification, 10% hydrogen gas (final concentration) was added to the headspace of the final set of microcosms. The effect of both ferric and ferrous iron was examined by adding 50 and 200 mg/L Fe^{2+} (as $FeSO_4 \cdot 7H_2O$) or 50 and 200 mg/L Fe^{3+} (as $FeCl_3 \cdot 6H_2O$) to microcosms. Although ambient Cinnamon Gulch sediment slurries were used as controls, it should be noted that at pH 3.5 or higher, added ferric iron precipitated.

Water Chemistry Analyses

Nitrate samples were filtered through 0.1μm Millipore filters, preserved by freezing and analyzed using a Dionex 500 Ion Chromatograph with carbonate-buffered eluent (1.8 mM Na_2CO_3/ 1.7 mM $NaHCO_3$, pH 7.5) at a flow rate of 2.00 mL/min through an IonPac AS4A analytical column and an AG4A guard column (Dionex Corp., Sunnyvale, CA). Ammonium samples were filtered through 0.1 μm Millipore filters, preserved with concentrated sulfuric acid to a final pH of 2 and analyzed using a Dionex 300 Ion Chromatograph with a gradient method, using eluent ranging from

2.5 to 25mN sulfuric acid over the course of the run at 1.5 mL/min through a CS12A analytical column and a CG12A guard column. Nitrite samples were filtered through 0.1μm Millipore filters and frozen until analysis. Nitrite was reduced to nitric oxide with 0.5mL of 2% sodium iodide added to 4mL of glacial acetic acid, exposed to ozone and passed through a chemiluminescent nitric oxide detector (Sievers Analytical, Model NOA 280, Boulder, CO). DOC samples were filtered through ashed GF/F filters and analyzed using a Total Organic Carbon Analyzer (Shimadzu Corp. Model TOC-5050, Kyoto). For dissolved trace metals, stream water was filtered through 0.1μm Millipore filters, preserved with trace metal-grade hydrochloric acid and analyzed by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) (Liberty Series 2, Varian Corporation). Iron species (Fe(T) and Fe(II)) were measured using the FerroZine method [To et al. 1999].

First-order specific denitrification potential rate constants were calculated using the average of nitrate concentration data from the three replicate flasks, collected at 0, 24, 48 and 72 hours of incubation by linear regression to the model

$$\ln\left[\frac{N}{N_o}\right] = -k * t ,$$

where N is the average nitrate-nitrogen concentration (μmole/L), N_o is the average initial nitrate-nitrogen concentration (μmole/L), t is elapsed time (in days) and k is the specific first-order reaction constant (μmole-NO_3-N g-sediment^{-1} day^{-1}). The regression slope was divided by the average sediment mass per flask (dry weight) to calculate the specific first-order denitrification potential rate constant, k.

Results

Complete Denitrification Microcosms

Results from initial microcosm incubations with surface water only, surface water and sediment, and gamma-sterilized sediment and surface water showed that nitrate was only utilized in the surface water and sediment microcosm (results not shown). Ambient nitrate concentrations in these field sites averaged 36 μM, so nitrate was added to better assess the process of denitrification and the possible effects of increased nitrate through deposition. Initially, samples were taken at 2 and 4 hours after becoming anaerobic. Nitrate was utilized immediately in those microcosms, but those time points were eliminated from further studies to provide more sampling points near the end of the incubation when nitrate in some microcosms was utilized at a slower rate. Slurries of freshly collected sediment from the Pennsylvania Mine effluent stream demonstrated immediate and consistent nitrate utilization and nitrogen gas accumulation (Figure 4-2) when incubated with added nitrate. Nitrate was reduced to nitrogen gas stoichiometrically, indicating that the microbes present in the sediments were capable of complete denitrification.

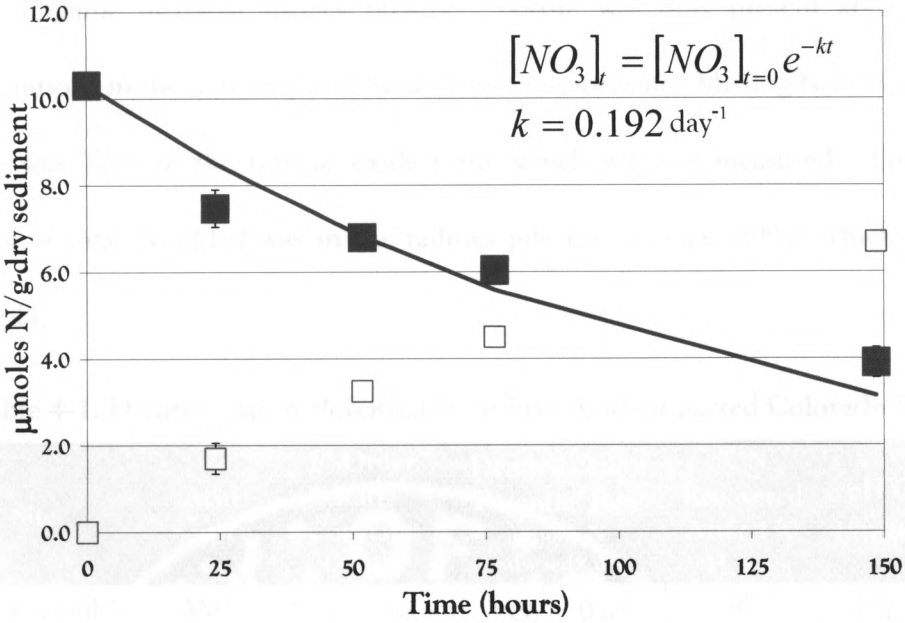

Figure 4-2. Nitrate (■) and nitrogen gas (□) concentrations during anaerobic incubation of Pennsylvania Mine sediment and the calculated first-order denitrification potential rate (–).
Error bars represent +/– standard deviation.

Complete denitrification to nitrogen gas occurred in microcosms from all five sites studied. First-order denitrification potential rate constants for each site were determined after 51 hours of incubation (Table 4-1). Cinnamon Gulch had the highest rate constant of 0.323 day^{-1}; the lowest was St. Kevin Gulch at 0.059 day^{-1}. Denitrifiers were active even in the tailings pile spring, which had the lowest initial pH of 2.60, but not the lowest rate constant. During the denitrification potential incubation, the pH in all microcosms increased. The largest pH increase occurred in the Snake River microcosms, with an initial pH of 4.54 and a final pH of 6.03.

After 150 hours, the total inorganic N (NO_3^-, NO_2^-, N_2, NH_4^+) in the microcosms was determined for mass balance purposes. No ammonium was detected at any time point in any of the microcosm samples. Nitrate and nitrogen gas were the

major inorganic nitrogen species present. Nitrite was only present at significant concentrations in the naturally acid Snake River microcosms. Missing N from the mass balance was likely in the nitrous oxide form, which was not measured. The lowest recovery of total N added was in the tailings pile microcosms (89%), which had the lowest pH.

Table 4-1. Denitrification Potential from Five Acid-Impacted Colorado Sites

Site	Acid Source	1st-Order Rate Constant [day^{-1}]	Denitrification Potential Rate $\frac{\mu moles\ N}{g\text{-}dw\ sed \times day}$	% N Recovered After 150 Hours	pH Change Initial→Final
St. Kevin Gulch	AMD	0.059±0.008	1.06 ± 0.03	97	4.20→4.74
Tailings Pile	AMD	0.143±0.003	1.52 ± 0.07	89	2.60→2.97
Pennsylvania Mine	AMD	0.192±0.010	1.15 ± 0.04	95	3.23→4.07
Cinnamon Gulch	AMD	0.323±0.008	0.38 ± 0.012	97	4.12→4.35
Snake River	Natural	0.172±0.026	0.50 ± 0.08	97	4.54→6.03

Iron increased greatly, from 20mg/L to 215mg/L in the Pennsylvania Mine site and from 0.4 to 13.5mg/L in the nearby Cinnamon Gulch site (Figure 4-3). With flask pHs from Pennsylvania Mine and Cinnamon Gulch greater than 3, the increase probably indicates production of soluble ferrous iron, Fe^{2+}. Iron concentrations in the tailings pile, St. Kevin Gulch and Snake River sites decreased during denitrification potential incubations.

Soluble heavy metals (Al, Cd, Co, Cr, Cu, Mn, Ni, and Zn) were measured during the denitrification potential incubations for flasks from all five sites (Figure 4-4). Aluminum decreased in all microcosms during the incubation, with the greatest decrease in the Pennsylvania Mine site. Cadmium decreased in the tailings pile spring site, increased in the Pennsylvania Mine and St. Kevin Gulch sites and remained at or

below the detection limit in both the Cinnamon Gulch and the Snake River sites. Chromium decreased in the tailings pile spring site, remained relatively constant in the Pennsylvania Mine site and was at or below detection limits in the remaining sites. Cobalt increased in all samples except the naturally acid Snake River site, where it decreased during incubation. Copper decreased significantly in the Pennsylvania Mine site and slightly in the St. Kevin Gulch site and remained at or below detection limit in the other sites. The concentration of manganese decreased overall in the tailings pile and Snake River sites and increased in the Pennsylvania Mine, Cinnamon Gulch and St. Kevin Gulch sites. Nickel concentrations remained relatively constant in all sites except in the Snake River site, where the concentration decreased to detection limit in the first 24 hours. Zinc concentrations remained relatively constant in all sites except the tailings pile site, where it decreased over the incubation period.

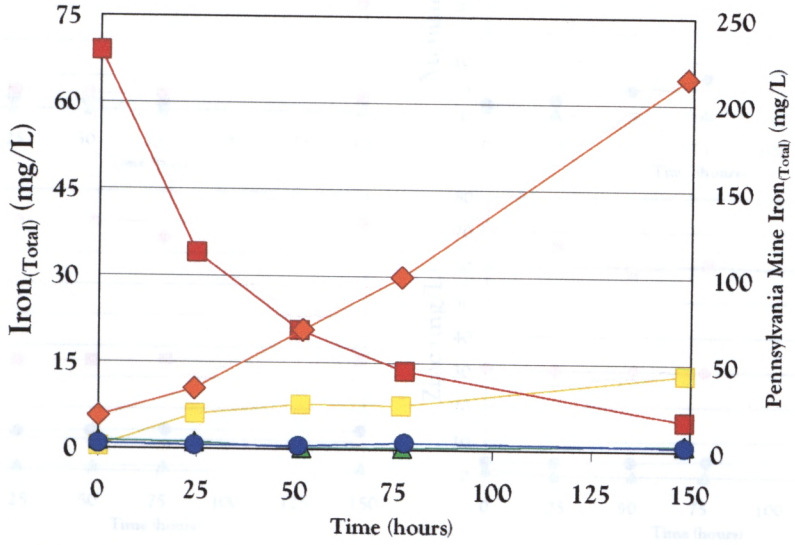

Figure 4-3. Dissolved total iron concentrations during denitrification from five acid-impacted Colorado Rocky Mountain sites. Data are averages of three replicate flasks. Error bars represent +/- standard deviation.

● St. Kevin Gulch, ■ Spring in tailings pile, ■ Cinnamon Gulch, ▲ Snake River, ◆ Pennsylvania Mine – Secondary Axis

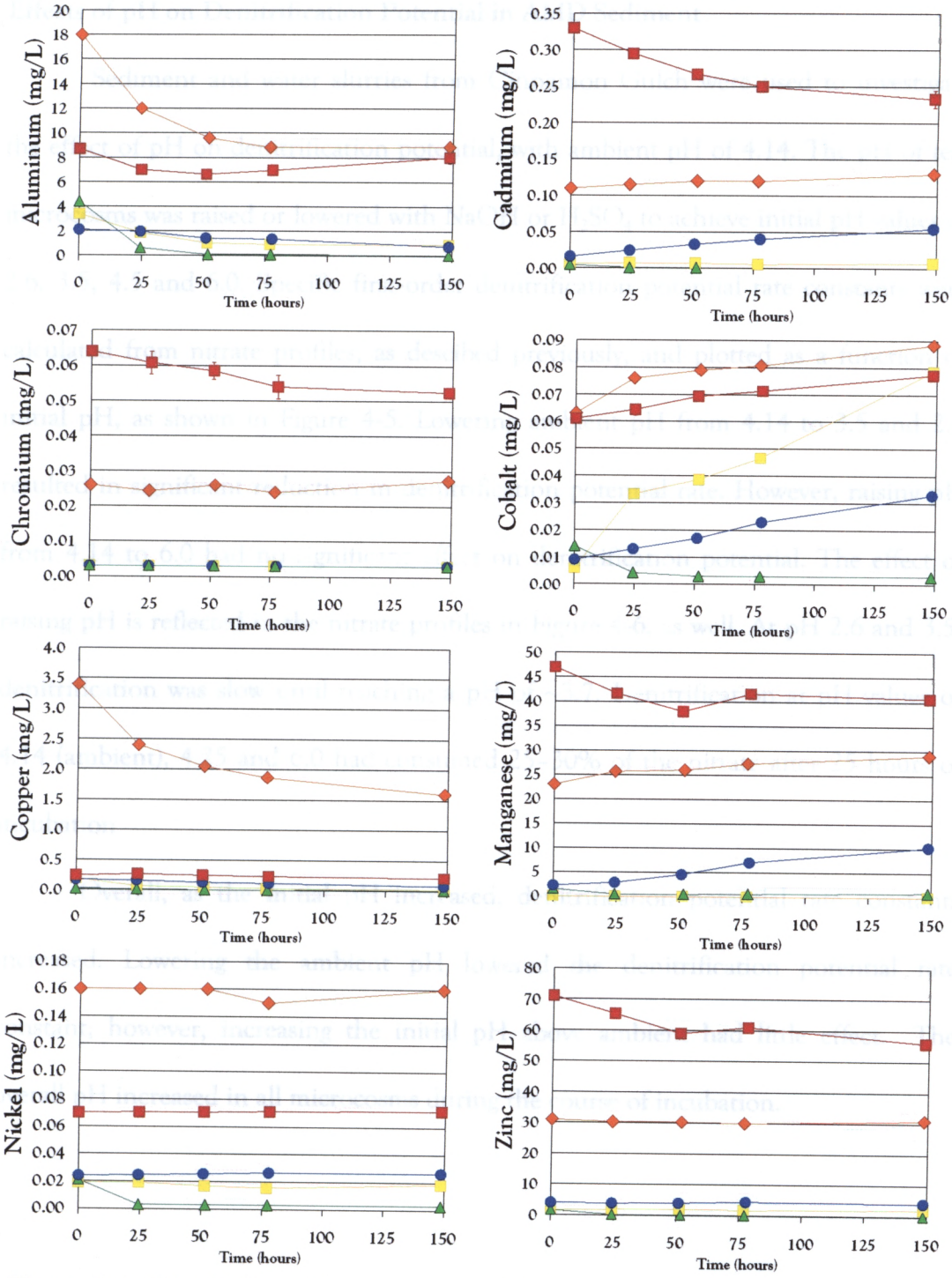

Figure 4-4. Dissolved metal concentrations during denitrification from five acid-impacted Colorado Rocky Mountain sites. Data are averages of three replicate flasks. Error bars represent +/- standard deviation.

● St. Kevin Gulch, ■ Spring in tailings pile, ◆ Pennsylvania Mine, ■ Cinnamon Gulch, ▲ Snake River

Effects of pH on Denitrification Potential in AMD Sediment

Sediment and water slurries from Cinnamon Gulch were used to investigate the effect of pH on denitrification potential, with ambient pH of 4.14. The pH of test microcosms was raised or lowered with NaOH or H_2SO_4 to achieve initial pH values of 2.6, 3.5, 4.5 and 6.0. Specific first-order denitrification potential rate constants were calculated from nitrate profiles, as descibed previously, and plotted as a function of initial pH, as shown in Figure 4-5. Lowering ambient pH from 4.14 to 3.5 and 2.6 resulted in significant reduction in denitrification potential rate. However, raising pH from 4.14 to 6.0 had no significant effect on denitrification potential. The effect of raising pH is reflected in the nitrate profiles in Figure 4-6, as well. At pH 2.6 and 3.5, denitrification was slow until reaching a pH of ~3.7. Denitrification at pH values of 4.14 (ambient), 4.25 and 6.0 had consumed 25–30% of the nitrate after 25 hours of incubation

Overall, as the initial pH increased, denitrification potential rate constants increased. Lowering the ambient pH lowered the denitrification potential rate constant; however, increasing the initial pH above ambient had little effect. The overall pH increased in all microcosms during the course of incubation.

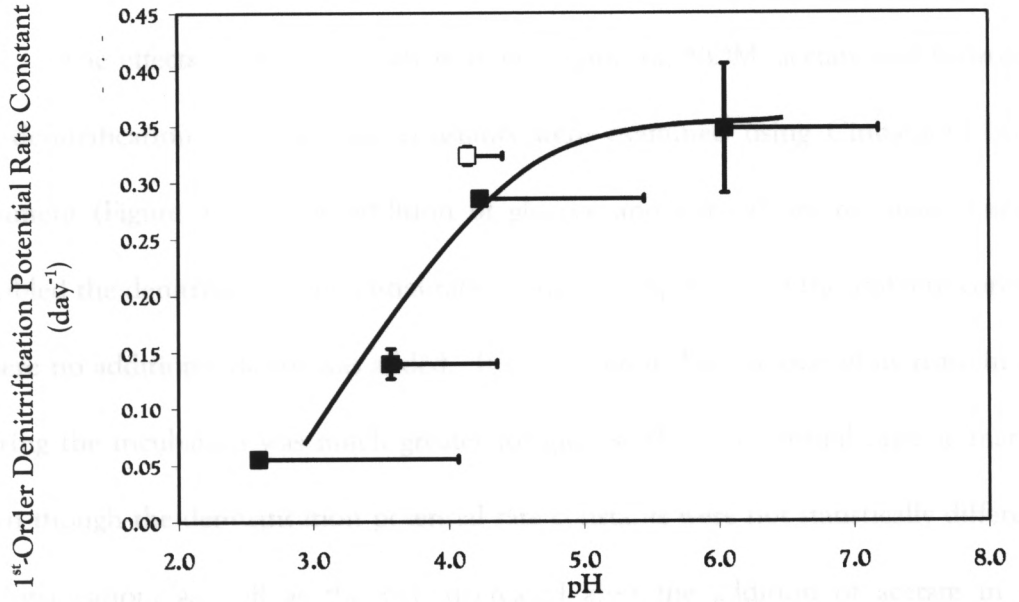

Figure 4-5. First-order denitrification potential rate constants in AMD-impacted Cinnamon Gulch sediment vs. initial microcosm pH. □ represents the control. Rate constants were determined using 0- to 48-hour time points. Y-axis error bars are the +/- standard deviation from the rate calculation, and X-axis error bars are the range of pH during the 165-hour denitrification potential incubation.

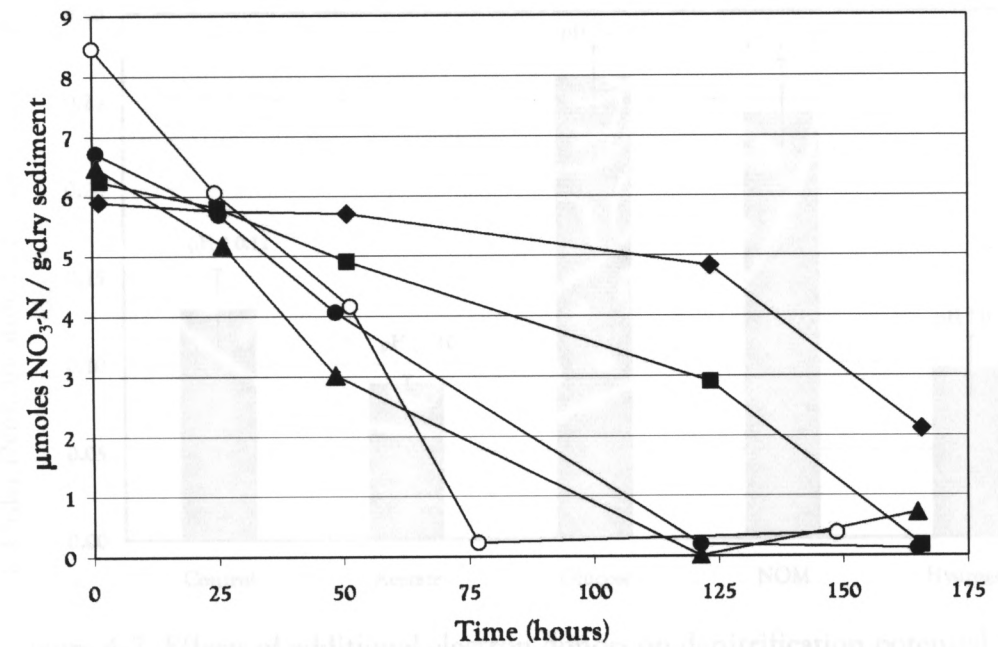

Figure 4-6. Nitrate concentration in AMD-impacted Cinnamon Gulch sediment during anaerobic microcosm incubations with adjusted pH. Data points are averages.

◆ pH 2.6, ■ pH 3.5, ○ Control (pH 4.14), ● pH 4.25, ▲ pH 6.0

Error bars representing ± standard deviation for triplicates are smaller than the sample.

Effects of Potential Electron Donors on Denitrification Potential in AMD Sediment

The effects of possible electron donors (glucose, NOM, acetate and hydrogen) on denitrification potential rate constants were examined using Cinnamon Gulch sediment (Figure 4-7). The addition of glucose and natural organic matter nearly doubled the denitrification potential rate constant compared with the ambient control, where no additional donor was added. It is of interest that the overall increase in pH during the incubation was much greater for glucose than for natural organic matter, even though the denitrification potential rate constants were not statistically different. Denitrification, as well as the pH, decreased after the addition of acetate in the microcosms. The addition of 10% H_2 to the headspace had no statistically significant effect on the denitrification potential rate compared with the ambient controls.

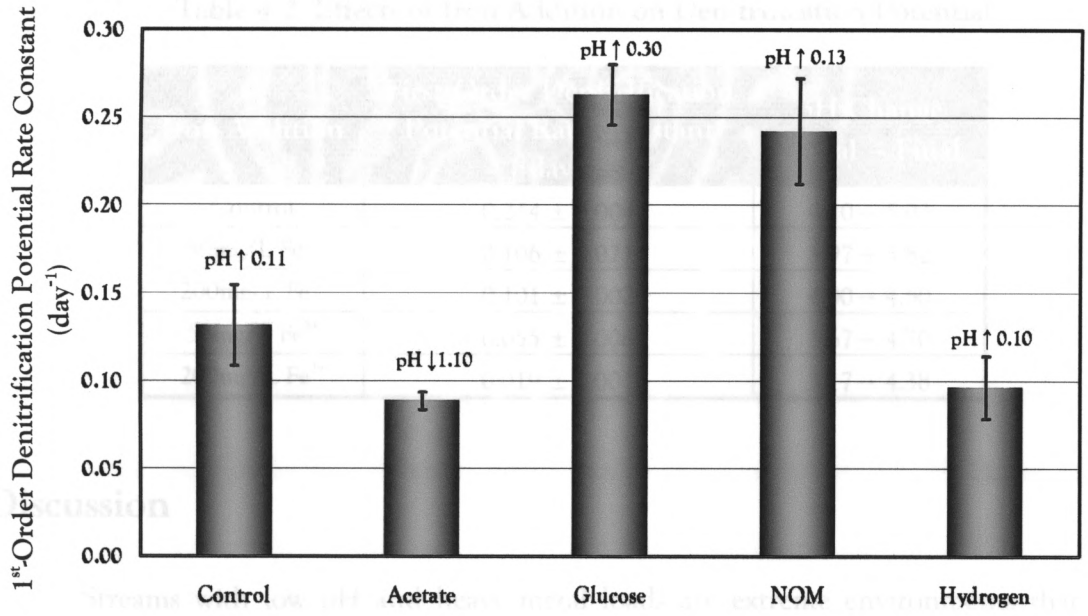

Figure 4-7. Effects of additional electron donors on denitrification potential in AMD-impacted Cinnamon Gulch sediment. Rate constants were determined using 0- to 48-hour time points. Error bars represent +/- standard deviation. pH changes are after 193 hours of incubation.

Effects of Iron Addition on Denitrification Potential in AMD Sediment

In general, added iron resulted in lower denitrification activity in Cinnamon Gulch sediment (Table 4-2). Background concentrations of dissolved total iron in the control sample were 24.81 mg/L, with 93% as Fe^{2+}. The addition of 50 and 200 mg/L of Fe^{2+} had the same relative effect on denitrification potential, decreasing the specific rate constants by about 50%. The pH increased during both tests; in the 50mg/L Fe^{2+}, the increase was twice that of the control. The addition of Fe^{3+} also produced decreased denitrification potential rates: by ~50% for 50 mg/L Fe^{3+} added and by 80% for 200 mg/L Fe^{3+} added. However, the pH in the microcosms that had Fe^{3+} added increased approximately the same amount as did the control. The addition of Fe^{3+} depressed the initial pH of the microcosms.

Table 4-2. Effects of Iron Addition on Denitrification Potential

Iron Addition	First-Order Denitrification Potential Rate Constant [day^{-1}]	pH Change Initial → Final
Control	0.214 ± 0.004	4.10→ 5.03
50mg/L Fe^{2+}	0.106 ± 0.021	3.97→ 5.82
200mg/L Fe^{2+}	0.101 ± 0.002	4.00→ 4.60
50mg/L Fe^{3+}	0.055 ± 0.008	3.67→ 4.70
200mg/L Fe^{3+}	0.019 ± 0.001	3.17→ 4.38

Discussion

Streams with low pH and heavy metal loads are extreme environments that represent a challenge for survival for all forms of life [Madigan 2000]. Studies have shown that AMD environments have low biological diversity as compared with pristine environments [Niyogi et al. 1999]. The objective of this study was to look at one

component of stream ecosystem function—denitrification. Denitrification is important for two reasons: the removal of anthropogenic fixed nitrogen from the system as N_2 and the generation of alkalinity [Postma et al. 1991], the latter of which may mitigate the impact of AMD. Results from this study demonstrated that denitrifying microorganisms are indeed present and active at AMD-impacted sites. Additional environmental factors were also examined to explore possible controls on the process in these sites.

Under basic conditions (pH>8), nitrate can be reduced abiotically to ammonium or nitrite in the presence of Fe^{2+}, and nitrogen gas can be reduced abiotically in the presence of aluminum [Fanning 2000] but not in acidic environments, such as the ones studied here. Nitrate has also been shown to be abiotically reduced to ammonium in the presence of green rust ($Fe^{2+,\ 3+}$ hydroxides) [Chr et al. 1996]. Abiotic controls used in this experiment demonstrated that biological denitrification was the main mode of nitrate reduction in these systems; nitrate reduction to ammonium was not detected. Nitrite, an intermediate in the denitrification pathway, did not appear in any appreciable concentrations in these microcosm studies—perhaps because nitrite was rapidly converted to NO or N_2O via the denitrification pathway. However, nitrite can be very reactive at low pH [Bollag et al. 1973], particularly in the presence of Fe^{3+} and light [Zhang and Bartlett 2000]. The end product for this reaction is NO_2 (gas), not N_2. However, in this study, the amount of nitrite reduced by light and Fe^{3+} was small, because the major end product associated with nitrate reduction was N_2.

Many investigators have reported that denitrification is inhibited by low pH [Blosl and Conrad 1992, Christensen 1985, Nagele and Conrad 1990a, b]. Studies examining denitrification in acidic soils have shown that N_2O is the major end product [Nagele and Conrad 1990a]. Results from the research presented here show that N_2 is the major end product in the reduction of nitrate, indicating that pH does not inhibit complete denitrification in AMD sediments. Nitrate was immediately reduced to nitrogen gas in these microcosm studies, suggesting that denitrifiers in AMD streams have adapted to the extreme environment and express the full suite of enzymes necessary to reduce nitrate to N_2, thus obtaining the maximum energy. The pH of the microcosms also increased during denitrification for all sites tested, suggesting that removal of one contaminant, nitrate, may help reduce the effects of another, AMD.

All five acid-impacted sites showed denitrification potential at ambient pH ranges from 2.6 to 4.54. The first-order rate constants for denitrification potential in these experiments were higher than those reported in acidic soils and uncontaminated soil environments. In an acidic spruce forest soil, the accumulation of N_2O from the acetylene block technique for soil horizon denitrifying enzyme activity ranged from 19.8 to 304.4 ng N_2O-N g^{-1} dry-wt hr^{-1} (0.034 to 0.522 μmoles N_2O-N g^{-1} dry-wt soil day^{-1}) [Henrich 1997], which is slightly lower than the range of denitrification potential rates found in the AMD sediments. nitrate consumption rates in an acidic coniferous soil under anaerobic conditions ranging from 45.6 to 1252.6 μg N g dry weight-$sediment^{-1}$ incubated for 24 hours (3.3 to 89.5μmoles NO_3-N g^{-1} dry-wt sediment day^{-1}) [Laverman et al. 2001], which is approximately 2 to 60 times higher than the rates

reported here (Table 4-1). In a study examining 29 different soils that used the acetylene block technique as a measure of denitrification, rates ranged from 0.002 to 2.65ng N_2O-N g^{-1} dry weight -soil hr^{-1} (0.0326 to 4.5nmoles N_2O-N g^{-1} dry-wt day^{-1}) [Bollmann and Conrad 1997], which is more than an order of magnitude less than in AMD sediments. The AMD denitrification potential rates compared with other soils indicate that the low-pH, heavy metal-laden sediments are capable of higher denitrification potential than soils with neutral pH but lower than acidic forest soils. With nitrate deposition increasing in the Rocky Mountains [Williams 2000], the denitrification capacity of acidified streams is encouraging.

Examining the effects of pH on the denitrification process, it appears that the community of microbes in the tailings pile spring (pH 2.6) has a higher capacity for denitrification than some other stream sediments with higher pH. Within one site (Cinnamon Gulch), however, there does seem to be an increase in activity with increasing pH up to a certain plateau, where a further increase in pH does not stimulate activity. Together, these data suggest that within a specific site, the microbial community has adapted denitrification mechanisms to the specific pH seen at that site.

Denitrifying microorganisms at these sites appear to have adapted to high concentrations of metals. Many of the metal concentrations decreased during denitrification incubations, likely the result of increasing pH, which caused the precipitation of metals. Heavy metals are often toxic to organisms. In streams with high concentrations of these metals, such as AMD-impacted streams, the extent of diversity is affected [Niyogi et al. 1999]. The decrease in metal concentrations during

denitrification may be important when considering remediation alternatives and restoration for AMD-impacted streams.

Results from electron donor addition experiments indicated that denitrification may be substrate-limited in these sediments. Many mountain streams experience peak nitrate concentrations during snowmelt due to nitrogen deposition in snow [Brooks and Williams 1999]. DOC concentrations are also high during snowmelt [Boyer et al. 2000]. Rapid denitrification might be expected during this time of year, because both the electron supply and the electron acceptor concentrations peak. Alternately, dissolved organic carbon has also been shown to sorb to sediments in acid-impacted streams [McKnight et al. 2002], and this carbon may be a year-round source of electron donors for heterotrophic denitrifiers living within these sediments.

Many studies have reported that carboxylic acids, especially acetate, are widely used by denitrifying bacteria. In a study with groundwater sediment, the preferred electron donor tested was acetate, the degradation of which generated bicarbonate [Devlin 2000]. A similar result in AMD sediments could cause an increase in pH. However, results from the acetate addition in this study decreased the denitrification potential and the pH, indicating that acetate is not a likely donor for denitrification in AMD sediments and that any acetate degradation that did occur had an antagonistic effect on ambient pH.

Given that carbon is often limited in AMD systems, it is important to consider other non-organic electron donors for denitrification. Another environment in which carbon is often limiting is groundwater systems. Studies have examined hydrogen,

elemental iron, Fe^{2+}, pyrite and sulfur compounds as possible electron donors for denitrification in the subsurface [Devlin 2000, Till 1998, Yamamoto-Ikemoto 2000]. Elemental sulfur can also be used as an electron donor in the denitrification process [Oh et al. 2002, Yamamoto-Ikemoto 2000]. However, in reactions with thiosulfate and elemental sulfur, while nitrate is reduced to nitrogen gas, hydrogen ions are generated [Devlin 2000]. This reaction would lower the pH, which is not what occurred in the tested AMD systems when an exogenous electron donor was supplied. The reaction in the test sites might suggest that sulfur is not a significant electron donor for denitrification in AMD sediments.

The denitrification potential rate in the presence of added hydrogen was not statistically different from the control. Researchers have reported that hydrogen-utilizing bacteria are mixotrophs that, in the presence of biodegradable organic carbon, will preferentially use the carbon. It is likely that natural organic matter on sediments was used during the ambient denitrification observed in microcosms, and a sufficient supply of NOM may have suppressed hydrogen utilization. Also, highly aerated streams with shallow sediments may be an unlikely environment for H_2 production, reducing the opportunity for a hydrogen-utilizing population to develop.

Pyrite (FeS_2) can also be an electron donor for denitrification and is a proton-consuming reaction, with Fe^{2+}, SO_4^{2-} and N_2 as the end products [Devlin 2000, Postma et al. 1991, Till 1998]. This may be important to consider, because pyrite is ubiquitous in AMD streams. In a study of groundwater sediments by Postma and others [Postma et al. 1991], pyrite appeared to be the main electron donor in the reduction of O_2 and

NO_3^-, even though organic matter was more abundant. This could be a factor in the Pennsylvania Mine and Cinnamon Gulch sediments, because concentrations of total dissolved iron, as well as the pH, increased during the incubations. Dissolved iron did not increase in the other microcosms, but this could be a result of precipitation of iron complexes at higher pHs. There was an increase in sulfate concentrations (data not shown) during all denitrification potential incubations, which could indicate that pyrite may be an important source of electron donors in this process when carbon is limited.

Experiments were also conducted to examine the effects of additional Fe^{2+} and Fe^{3+} on denitrification in AMD sediments. Fe^{2+} could potentially be used as an electron source in autotrophic denitrification where nitrate is reduced to nitrogen gas and FeOOH and hydrogen ions are generated [Devlin 2000]. Fe^{2+} was not expected to be a significant donor for denitrification in these systems because of the generation of acid. However, it is not clear why additional Fe^{2+} had a negative effect on denitrification. Fe^{3+} can be a competitive electron acceptor with nitrate when both are present [Madigan 2000], which could explain the decrease in denitrification potential rates when Fe^{3+} was added.

Conclusions

Denitrifying microorganisms are present and active in acid-impacted stream sediments. Reduced nitrate at rates from 0.059 to 0.323 μmole-N/g-sediment/day in anoxic flasks supplements with 5 mg/L NO_3-N with initial pH from 2.6 to 4.54 pH increased during denitrification in all microcosms from 0.4 to 1.5 units, with the

highest increase at an initial pH of 4.54 (1.5 units) and the lowest at a pH of 2.6 (0.4 units). The denitrification potential rates at pH values below 3.7 were lower than those at 4.1. However, increasing test flask pH to 6.0 did not result in increased denitrification. Addition of glucose or natural organic matter stimulated denitrification, suggesting that the process is substrate-limited in oligotrophic mountain streams. However, acetate inhibited denitrification in low pH conditions. Denitrification potential rates were reduced by 50–80% with the addition of ferric or ferrous iron, with ferric iron causing the greatest effect.

Little was known about nitrogen cycling in AMD environments to this point. From the results presented here, it is clear that nitrogen cycling, in particular denitrification, may be an important process that has been overlooked in AMD environments. Denitrification may have the potential to be used as a remediation strategy for AMD-impacted streams. Stimulating the community with glucose is a method that warrants further study, because this carbon source not only increases denitrification potential rate but also increases pH more than the other sources of carbon examined. Increasing the presence of heterotrophs has been shown to be a viable remediation option of tailings piles [Marchand 2000]. More research needs to be conducted to examine how increased carbon and heterotrophs might affect downstream processes, such as denitrification.

Chapter 5:

Effects of Water Chemistry on Denitrification Potential in Snowmelt-Dominated, Acid-Impacted Mountain Streams

Abstract

Many stream systems in the Rocky Mountains are affected by acid rock or acid mine drainage. Until now, little was known about nitrogen cycling in acid-impacted streams. Some of these same streams are also vulnerable to increasing nitrate concentrations via increasing nitrogen deposition. The hydrograph of mountain streams is dominated by snowmelt, which has been shown to bring with it a pulse of nitrate, DOC and, in AMD-impacted sites, a flushing of metals. This study examined the ability of acid-impacted streams to support denitrification, the nitrate reduction to nitrogen gas, thus removing anthropogenic inputs of excess nitrate from the ecosystem. Nitrate at concentrations from 30 to 60 μM (0.4 to 0.8 mg/L NO_3-N) was the primary nitrogen species measured in all the streams. Lower concentrations of nitrite, ammonium and nitrous oxide suggested that there is a biogeochemical nitrogen cycle in these acidic, heavy metal-laden streams. To determine if a viable population of denitrifying microorganisms was present and active throughout the year, sediment and water samples taken during snow cover, snowmelt and summer flow conditions were incubated in anoxic microcosm flasks in the presence of nitrate. Selected streams included mine effluent, a higher-order AMD-impacted stream, a naturally acidic

stream and, for comparison, a pristine, near-neutral pH stream, all from nearby alpine catchments. Denitrification potential rate constants measured in the laboratory were highest for samples taken during snow cover from all sites examined. Ambient pH and total dissolved metal concentrations were not the predominant factors influencing denitrifying activity. However, DOC concentrations correlated significantly to higher denitrification potential rate constants across all samples, indicating that heterotrophs dominated the denitrification process at low pH and that substrate availability influenced the denitrification potential rate. The presence of other inorganic nitrogen species, such as nitrite, ammonium and nitrous oxide, indicates that there is an active biogeochemical nitrogen cycle in these low-pH, heavy metal–laden streams. The capacity of these acidic high mountain watersheds to assimilate nitrate contaminants, subject to substrate availability and flow conditions, is important to consider when predicting the effects of increased nitrogen deposition.

Introduction

As the population in the western United States continues to increase, so does the need for clean water sources [Brooks et al. 2001]. Mountain watersheds contain the headwaters of regional surface water supplies for these increasing populations, and many of these streams are contaminated by acid mine drainage or, less frequently, natural acid rock drainage [EPA 1994, McKnight and Bencala 1990, McKnight et al. 2002, Sullivan et al. 1998, USFS 1993]. AMD results from pyrite (FeS_2) oxidation in waste-rock piles, or tailings, removed from the subsurface and left exposed to the

elements [Madigan 2000, Marchand and Silverstein 2002]. As precipitation from snow and rain infiltrate these piles, chemical and biological reactions generate sulfuric acid and soluble iron, which in turn solubilize other heavy metals from the rock surfaces. The acidic, metal-laden leachate from these tailings piles flows from mine tunnels and rock piles into streams and creates an extreme environment for life [Johnson 1998, Madigan 2000].

Many of these same watersheds are also impacted by increasing loads of nitrate through atmospheric deposition of nitrogen oxides resulting from combustion, with nitrate being the important constituent [Brooks and Williams 1999, Meixner and Bales 2003, Williams 2000]. Research conducted on the ability of alpine systems to process the excess loads has shown that nitrogen (nitrate) saturation, the capacity of the ecosystem to assimilate nitrogen inputs, is an increasing concern in many alpine and subalpine regions of the Rocky Mountains [Williams 2000]. The result of nitrate supersaturation is the export of nitrate to downstream surface and groundwater supplies. The process of biological denitrification, or the reduction of nitrate to nitrogen gas, is the primary mechanism of nitrogen removal from aquatic and soil systems [Tiedje 1994].

Until now, nitrogen cycling in AMD-impacted streams has not received much attention; rather, studies focused on the primary AMD contaminants: metals, sulfate and acidity. However, mountain watersheds are hydrologically dominated by snowmelt, which occurs in May or June and is accompanied by significant peaks in stream discharge along with dissolved organic carbon [Boyer et al. 2000] and nitrate

[Brooks and Williams 1999]. In addition, a recent study has shown that snowmelt flushes zinc from AMD-impacted streams [Brooks et al. 2001]. Combining these trends in contaminant transport, it was hypothesized that, during snowmelt, AMD-influenced streams could experience high concentrations of nitrate, DOC and metals simultaneously, which might affect the biogeochemistry of nitrogen cycling throughout the year, especially if toxic conditions inhibited the establishment of a nitrogen-cycling biota.

The optimal pH of denitrification has long been considered to be neutral to alkaline (6–8) [Simek et al. 2002]; likewise, it has been thought that the complete reduction of nitrate to nitrogen gas is often inhibited at lower pH [Glass and Silverstein 1998, Nagele and Conrad 1990a, b, Parkin 1985]. However, in experiments described in Chapter 4 of this dissertation, denitrification in sediment samples from acidic streams occurred at pH values as low as 2.6. The goal of the research reported herein was to evaluate the effect of hydrologic and selected water quality conditions on the denitrification potential in acidified streams. In particular, the snowmelt-dominated hydrograph and limited availability of carbon/energy substrates were chosen as factors likely to determine denitrification potential rates in four alpine streams—mine effluent, a higher-order stream with AMD-impacted tributaries, a naturally acidic mountain stream, and a pristine, near-neutral pH mountain stream—in adjacent watersheds that experienced comparable hydrologic and climatologic conditions during the sampling period.

Methods

Site Description

Water and sediment samples were collected from four sites near Keystone, Colorado, in 2003 (Figure 5-1) (see also Chapter 3: Regions I and II) during snow cover (April), peak snowmelt (early June) and summer flow conditions (late July). The US Geological Survey has a stream gage on the Snake River near Montezuma, Colorado. The 2003 hydrograph with sampling dates is shown in Figure 5-2, along with the average flow over the past 56 years.

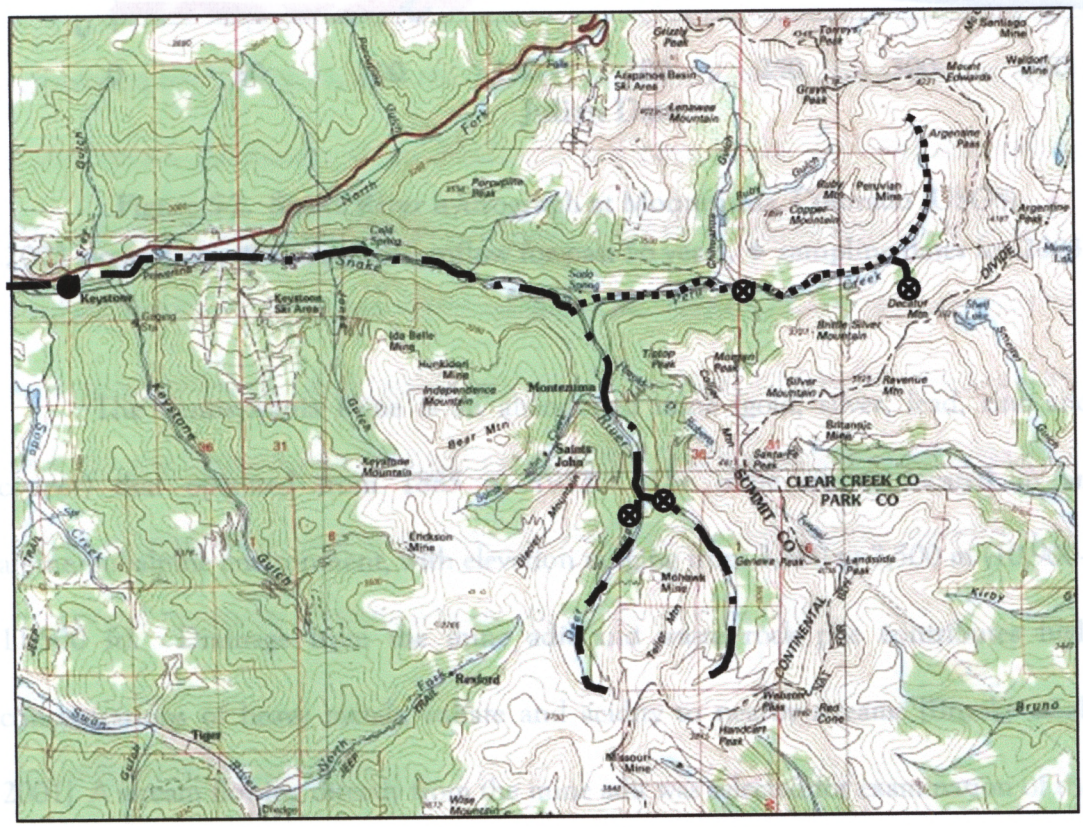

Figure 5-1. Map of sampling sites (⊗) near Keystone, Colorado

Pennsylvania Mine (—), Peru Creek (----), Snake River (—·—·—), Deer Creek (- - -)

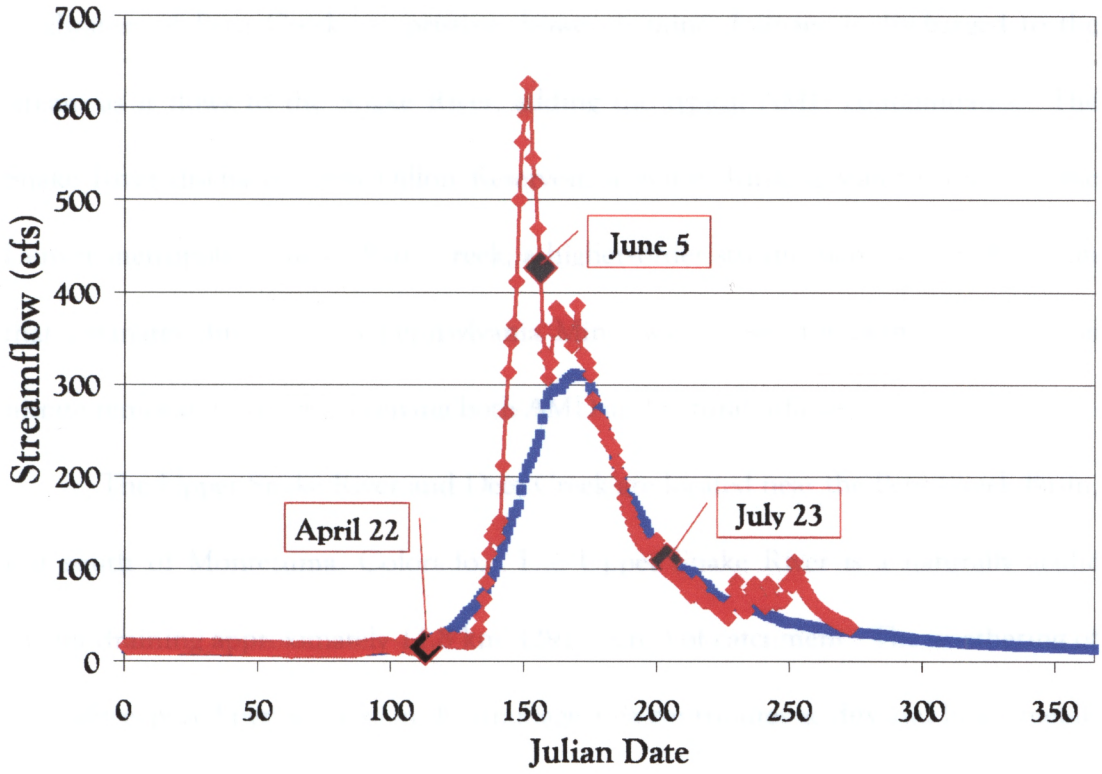

Figure 5-2. Snake River hydrograph at Montezuma, Colorado (USGS). ◆ 2003 streamflow, ■ average streamflow for past 56 years, ◆ sampling dates for 2003 study.

The Pennsylvania Mine is an abandoned mine (gold, silver, lead, zinc and copper) that operated from 1885 to 1953. The mine site is located in an alpine/subalpine environment, with elevation ranging from 3,300 to 3,500 m (10,900–11,500 ft). Drainage from the mine adit and waste rock pile contributes high concentrations of heavy metals, sulfate and acidity to nearby streams [Brooks et al. 2001]. A stream flowing directly from the mine adit was selected for examination of the fate of nitrate in drainage water and sediment. The Pennsylvania Mine drains into the second sampling site, Peru Creek, which is a second-order stream that collects drainage from several mines in the area from a catchment of 41.4 km^2 (10,156 acres). The

headwaters of Peru Creek are pristine; however, mine drainage is discharged to the stream as it flows to the Snake River, adding the typical AMD contaminants. The Snake River discharges into Dillon Reservoir, a major drinking water source for the Denver metropolitan area. Peru Creek, a higher-order stream than the runoff stream that emanates directly from Pennsylvania Mine, was chosen for examination of the nitrate removal in streams receiving both AMD and natural inflows.

The Upper Snake River and Deer Creek are located near the Peru Creek Basin, just south of Montezuma, Colorado. The Upper Snake River is a naturally acidic stream draining approximately 11.8 km^2 (2916 acres) of catchment. The weathering of naturally exposed pyritic rocks in the drainage area contributes acidity and heavy metals to the stream [McKnight et al. 2002]. The Upper Snake was used to compare biogeochemical nitrate reactions in a naturally acidified stream as opposed to in AMD-acidified streams. Deer Creek, a pristine mountain stream with circumneutral pH and low concentrations of metals and other ions, drains about 10.5 km^2 (2,595 acres) and flows into the Upper Snake [McKnight et al. 2002]. This stream was selected because it is close in geographic location to both the AMD-influenced and the naturally acidic streams and represents a pristine stream habitat [Boyer et al. 2000].

Denitrification Potential Assays

The potential for denitrification in the test streams was investigated using microcosm flasks in the laboratory with sediment and water samples from the streams within 24 hours of collection, and incubation with end-to-end rotation (6 rpm) under

anoxic conditions with added nitrate. Sediment was collected in Mason jars from three locations at each site and mixed to achieve an aggregate sample of the sediment community to account for anticipated heterogeneity of the sediment particles and biota in the streams. Sediment water mixtures were stored at 4°C for a maximum of 24 hours until the microcosm incubations were initiated. After mixing, the samples from each site were divided into triplicate microcosms as follows: 30mL of mixed sediment was added to a 125 mL flask along with 100 mL of surface water augmented only with 5 mg/L NO_3-N (357 μM) from KNO_3. Flasks were stoppered and purged with UPH helium for >20 minutes to attain anoxic conditions. Flasks were clamped to a horizontal shaft and rotated end-to-end (6 rpm) at room temperature for the duration of the experiment. Water and gas samples were collected at several time points during the incubations, with helium added to replace the volume removed. Nitrate, nitrogen gas and pH were measured at each time point and analyzed as described below.

First-order specific denitrification potential rate constants were calculated using the average of nitrate concentration data from the three replicate flasks, collected at 0, 24, 48 and 72 hours of incubation by linear regression to the model:

$$\ln\left[\frac{N}{N_o}\right] = -k * t$$

where N is the average nitrate-nitrogen concentration (μmole/L), N_o is the average initial nitrate-nitrogen concentration (μmole/L), t is elapsed time (in days), and k is the specific first-order reaction constant (μmole-NO_3-N g-sediment^{-1} day^{-1}). The

regression slope was divided by the average sediment mass per flask (dry weight) to calculate the specific first-order denitrification potential rate constant, *k*.

Water Chemistry

Stream water samples and snow, when present, were collected from each site and taken back to the lab for immediate preservation and analysis. pH was measured immediately with an Orion pH probe. Samples for anion determination were filtered through a 0.1 μm Millipore filter preserved by freezing and analyzed using a Dionex 500 Ion Chromatograph with carbonate- buffered eluent (1.8 mM Na_2CO_3/ 1.7 mM $NaHCO_3$, pH 7.5) at a flow rate of 2.00 mL/min through an IonPac AS4A analytical column with an AG4A guard column and a conductivity detector. Samples for ammonium determination were filtered through a 0.1 μm Millipore filter preserved with concentrated sulfuric acid to a final pH of 2 and analyzed using a Dionex 300 Ion Chromatograph with a gradient elution of 2.5 to 25mN sulfuric acid at 1.5 mL/min through a CS12A analytical column and a CG12A guard column and a conductivity detector.

Samples for nitrite analysis were filtered through a 0.1 μm Millipore filter and frozen until analysis. Nitrite was reduced to nitric oxide with 0.5mL of 2% sodium iodide added to 4mL of glacial acetic acid, which was exposed to ozone and passed through a chemiluminescent nitric oxide detector (Sievers Analytical, Model NOA 280, Boulder, CO). Nitrous oxide samples were collected in the field in 38mL serum bottles prepared with 200 μL of 12.5 N NaOH, sealed with thick butyl stoppers, crimped,

flushed with helium (for 15 minutes) and evacuated (to 15 psi for 5 minutes). In the field, triplicate samples were taken by adding 15 mL of surface water with a syringe into each serum bottle. Nitrous oxide in the headspace was measured using a gas chromatograph (GC Model 301, HNU Systems) equipped with a wide-range electron-capture detector (Model 140 BN, Valco Instruments Co., Inc.) using 5% methane/95% argon carrier gas; the instrument was calibrated with standard gas mixtures (Scott Specialty Gases, Plumsteadville, PA).

DOC samples were collected in the field in ashed amber bottles and filtered through ashed GF/F filters before storing at 4°C until analysis using a Total Organic Carbon Analyzer (Shimadzu Corp. TOC-5050, Kyoto) within three weeks of sample collection. For dissolved trace metals, stream water was filtered through 0.1 μm Millipore filters, preserved with trace metal-grade hydrochloric acid and analyzed by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) (Liberty Series 2, Varian Corporation).

Statistical Analysis

Pearson correlations were computed to examine relationships between water chemistry parameters and denitrification potential rate constants using the SPSS Database Software Program for Windows 10.0.5 [SPSS 1999].

Results

Denitrification Potential Assays

Seasonal effects on denitrification capacity in AMD-impacted streams were examined using laboratory microcosms. A major influence on mountain streams examined here was the impact of snowmelt. Assays were conducted with sediments collected during snow cover, snowmelt and summer flow conditions. Deer Creek, a pristine mountain stream, was used as a comparison for denitrification potential rates in acid-impacted streams, and the upper Snake River, which is naturally acidic, was used as a comparison between naturally occurring and anthropogenically caused acid contamination.

Results from initial microcosm incubations with surface water only, surface water and sediment, and gamma-sterilized sediment and surface water showed that nitrate was only utilized in the surface water and sediment microcosm (results not shown). Because ambient nitrate concentrations in these sites averaged 36 μM (0.5 mg NO_3-N/L), nitrate was added to better assess the process of denitrification and the possible effects of increased nitrate through deposition. Initially, samples were taken at 2 and 4 hours after becoming anaerobic. Nitrate was utilized immediately in those microcosms, but those time points were eliminated from further studies to allow more sampling points near the end of the incubation when nitrate in some microcosms was utilized at a slower rate overall. Nitrate was stoichiometrically reduced to nitrogen gas indicating that microbes present in the sediments were capable of complete denitrification. Nitrite and ammonium were not detected in any of the microcosms.

Mass balances conducted averaged ~95% recovery of nitrate added and nitrogen gas produced.

Overall, the pristine stream sediments had the highest denitrification potential rate constant; however, the naturally acidic site examined also demonstrated high denitrification activity (Figure 5-2). The denitrification potential rate constants for sediments from the pristine (Deer Creek), naturally acidic (Snake River) and AMD stream (Peru Creek) sites was highest during snow cover, with rates decreasing as the season progressed. Measurable denitrification activity was observed in all acid-impacted sediments during the three seasons examined. The Pennsylvania Mine sediment had the highest rate constant during snowmelt, with declining rates in sediment collected during snow cover and summer flow conditions. The rate constants in the two AMD sites (Pennsylvania Mine and Peru Creek) during the summer flow conditions were not statistically different, based on standard deviation.

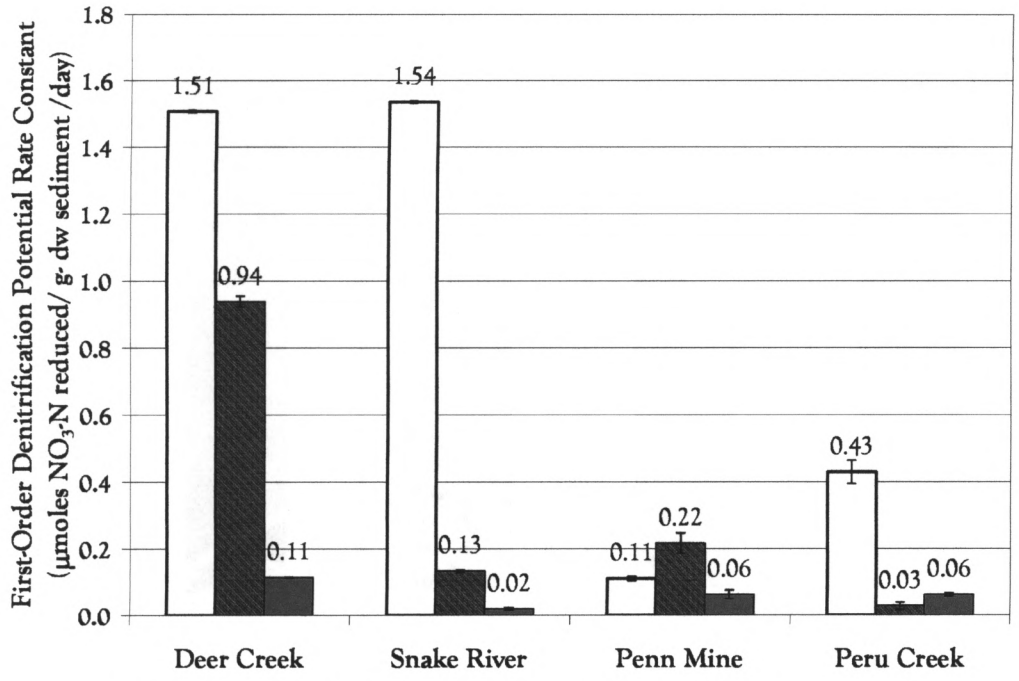

Figure 5-3. First-order denitrification potential rate constants in stream sediments dw = dry weight; error bars represent ± standard deviation.

□ Snow cover ▨ Snowmelt ■ Summer)

Water Chemistry

The pH of the acidic streams ranged from 3.05 to 5.64 throughout the sampling period (Figure 5-4), with the pristine stream (Deer Creek) remaining consistently more circumneutral at 6.21 to 6.36. In the naturally acidic Snake River site, pH was highest during snowmelt. The Pennsylvania Mine site and the Peru Creek site showed opposite pH trends, with the Pennsylvania Mine site pH highest during snow cover at 4.67 and decreasing to 3.05 in summer and the Peru Creek site pH increasing during the sampling period, from 3.74 during snow cover to 5.64 in summer.

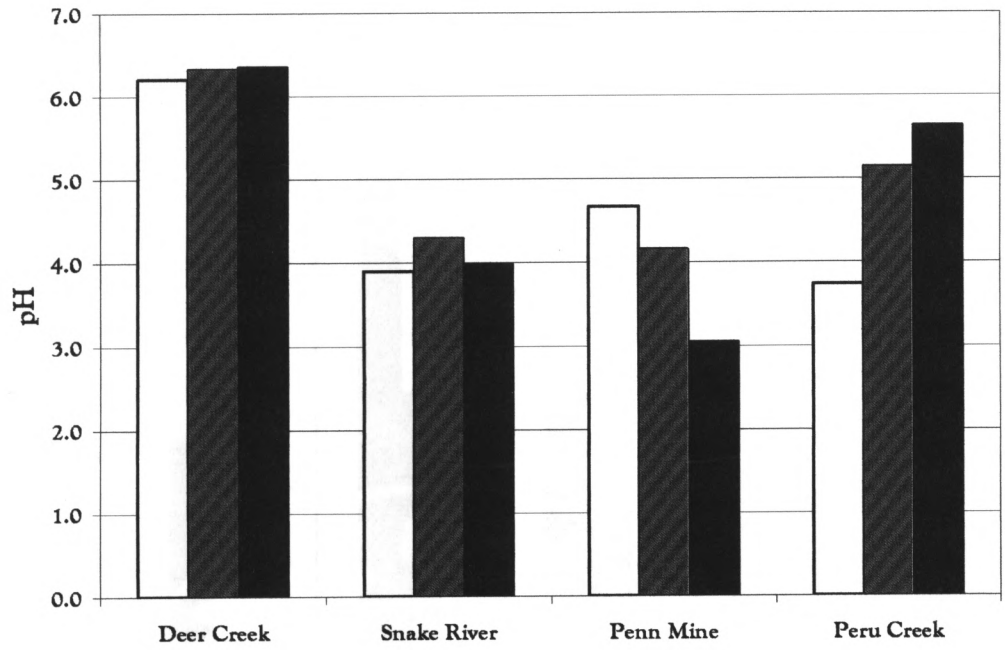

Figure 5-4. Stream Water pH

□ Snow cover ▨ Snowmelt ■ Summer

DOC concentrations were low at all sites (75.7 to 333.9 μM (0.9 to 4.0 mg/L)) (Figure 5-5), with the highest concentrations in the pristine stream (Deer Creek). Both Deer Creek and Snake River had the same seasonal DOC concentration trend; highest during snow cover (4.0 and 2.7 mg/L, respectively), decreasing from snowmelt (3.6 and 1.9 mg/L, respectively) to summer flow conditions (1.9 and 1.0 mg/L, respectively). DOC concentrations in the snowpack during snow cover and snowmelt (2.6 and 1.6 mg/L, respectively) were lower than those in Deer Creek and the Snake River and higher than in the AMD-impacted sites (Peru Creek and Pennsylvania Mine) during the same period. During snowmelt, at approximately the same value as the snowpack (1.5 mg/L), Peru Creek had the lowest DOC concentration, whereas Pennsylvania Mine had the highest.

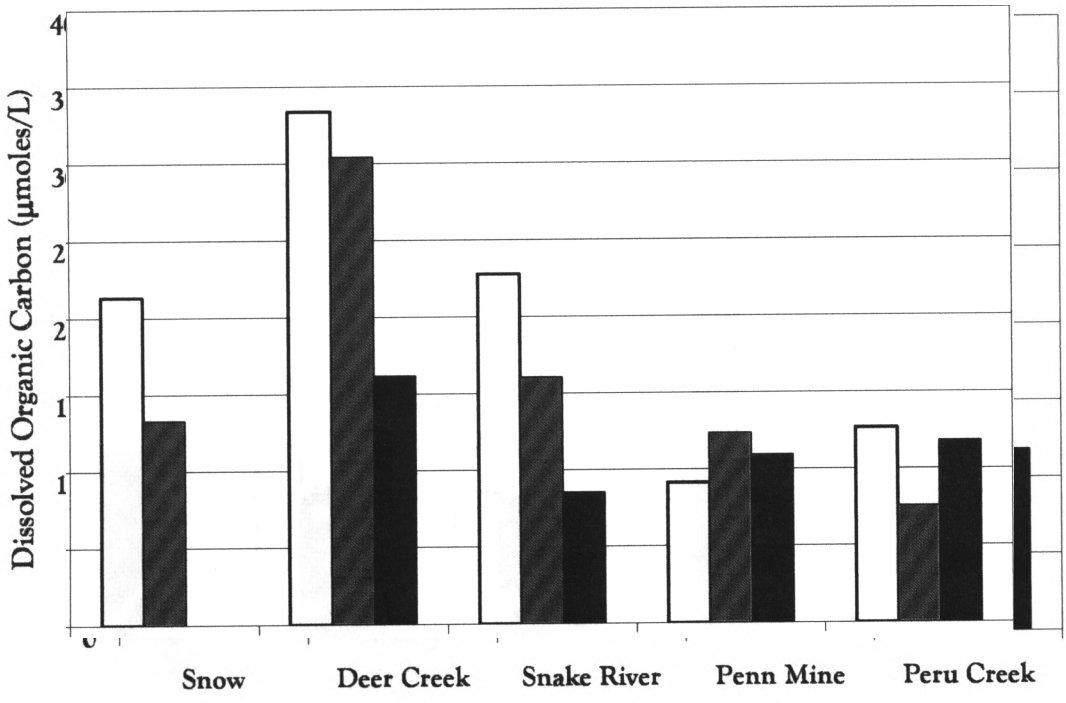

Figure 5-5. Concentration of dissolved organic carbon in snow and stream water

□ Snow cover ▨ Snowmelt ■ Summer

Figure 5-6 shows stream nitrate concentrations at all field sites and in the snowpack during the sampling period. Nitrate concentrations averaged 36.0 (±14.0) µM over all the stream samples. Nitrate concentrations in the snow were very similar to those measured in the stream samples, indicating that stream nitrate was highly influenced by snowpack. The low standard deviation of the stream nitrate data supports this observation. Nitrate in both Deer Creek and Snake River was highest during snow cover and decreased slightly during snowmelt and summer. Nitrate concentrations varied most in the Pennsylvania Mine site, ranging from below detection—< 0.07 mg-N/L (<5 µM)—during snow cover to 0.87 mg-N/L (62.1µM) during snowmelt. Nitrate was highest at both AMD-impacted streams during

snowmelt, at values higher than the nitrate measured in the snowpack and in the pristine and naturally acidic streams.

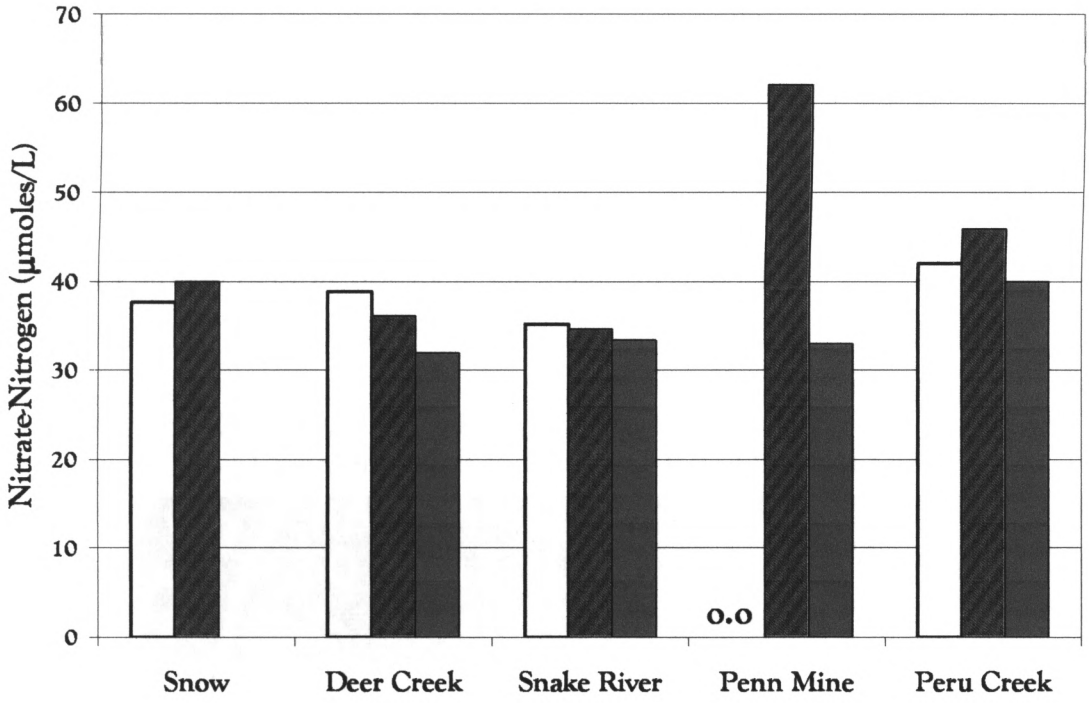

Figure 5-6. Concentration of nitrate in snow and stream water

□ Snow cover ▨ Snowmelt ■ Summer

Table 5.1 presents data for soluble nitrogen species other than nitrate (nitrite, nitrous oxide and ammonium) and sulfate. Ammonium was only present in the stream water during snow cover. Snowpack samples had the highest concentrations of 0.19 mg-N/L (13.4 µM) in late April and 0.1 mg-N/L (7.11µM) during snowmelt in early June. Nitrite concentrations in the streams were much lower than the concentration in the snow; stream nitrite concentration across all samples ranged from 0.08 to 0.56 µg-N/L (6.2 to 40.1 nM), compared with the snow nitrite concentration from 0.9 to 1.8 µg-N/L (64.7 to 132 nM). Nitrous oxide concentrations were low in these sites, and all

sites showed the lowest concentrations during summer conditions. Nitrous oxide concentrations were highest in Pennsylvania Mine during snowmelt, which is also when nitrate was highest. Sulfate concentrations in the pristine stream remained low throughout the season. In the acidic streams, the sulfate concentration tended to be inversely related to the pH; as pH increased, sulfate concentrations decreased, and vice versa. During snowmelt, sulfate concentrations decreased at all sites, suggesting that sulfate, a product of pyrite oxidation, was diluted by snowmelt.

Table 5-1. NO_2^-, N_2O, NH_4^+ and SO_4^{-2} Concentrations in Stream Water During Snow Cover, Snowmelt and Summer (DL: Detection Limit)

	Snow		Deer Creek			Snake River			Pennsylvania Mine			Peru Creek		
			Pristine			*Naturally Acidic*			*Direct Mine Effluent*			*AMD-Impacted*		
	4/22	6/5	4/22	6/5	7/23	4/22	6/5	7/23	4/22	6/5	7/23	4/22	6/5	7/23
NO_2^- nM DL= 10	64.7	132	39.1	40.1	38.7	7.7	6.2	6.2	6.2	6.2	6.2	6.2	11.8	34.9
N_2O nM DL=1	NA	NA	11.2	12.8	1.5	14.3	9.0	6.3	9.5	16.3	5.2	9.6	3.0	2.9
NH_4^+ µM DL=5	13.4	7.11	1.10	0.00	0.00	7.33	0.00	0.00	6.99	0.00	0.00	0.00	0.00	0.00
SO_4^{2-} mM DL=0.005	0.05	0.04	0.25	0.12	0.12	1.35	0.42	0.68	7.64	3.27	16.4	1.07	0.44	0.48

Metal concentrations, shown in Table 5-2, were highest in the Pennsylvania Mine site, which represents direct mine effluent. As would be expected, the concentration of all heavy metals measured was highest in Pennsylvania Mine effluent, irrespective of season, and the lowest levels of heavy metals were measured in Deer Creek during the three sampling times. In any given stream, metal concentrations were

typically lowest during snowmelt, which is contrary to the flushing of metals seen in other studies [Brooks et al. 2001, August et al. 2002], suggesting the importance of dilution with higher pH water. In the naturally acidic stream (Snake River) and the higher-order AMD-impacted stream (Peru Creek), metal concentrations were highest during snow cover and lowest during summer.

Table5-2. Trace Metal Concentrations in Stream Water During Snow Cover, Snowmelt and Summer

Metal	Snow		Deer Creek			Snake River			Pennsylvania Mine			Peru Creek			Detection Limit
			Pristine			*Naturally Acidic*			*Direct Mine Effluent*			*AMD=Impacted*			
mg/L	4/22	6/05	4/22	6/05	7/21	4/22	6/05	7/21	4/22	6/05	7/21	4/22	6/05	7/21	mg/L
Zn	0.050	0.039	0.079	0.015	0.021	1.000	0.266	0.545	24.290	13.471	72.570	2.160	0.701	0.847	≤0.003
Cd	≤DL	0.002	≤DL	≤DL	≤DL	≤DL	0.002	≤DL	0.104	0.060	0.466	0.007	0.002	0.004	≤0.002
Ni	≤DL	≤DL	≤DL	≤DL	≤DL	0.040	≤DL	0.018	0.131	0.076	0.337	0.017	≤DL	≤DL	≤0.014
Mn	0.032	0.013	0.095	0.015	0.020	1.652	0.420	0.864	28.680	12.960	67.120	1.965	0.741	0.821	≤0.002
Fe	0.307	0.026	0.137	0.047	0.064	0.958	0.530	0.786	14.506	5.116	99.340	0.261	0.042	0.056	≤0.005
Cu	0.011	0.009	0.003	0.005	0.011	0.030	0.011	0.016	1.458	1.603	11.384	0.191	0.075	0.098	≤0.002
Al	0.073	≤DL	0.011	0.013	0.010	7.402	1.899	3.361	12.131	12.731	56.770	2.495	0.417	0.075	≤0.006

* Arsenic and gold were below detection limits in all samples.

Chromium and lead were only detected in Pennsylvania Mine in July–0.007 mg/L and 0.064 mg/L, respectively.

Statistical Correlations

Correlation analysis was used to examine relationships between denitrification potential rates measured in sediment microcosms in the laboratory and those measured in seasonal stream water quality conditions. Using only data from the acidic streams (Pennsylvania Mine effluent, Peru Creek and Snake River), the denitrification potential rate constant had the strongest positive correlation with DOC concentration in the stream water with an R^2 value of 0.77 (Figure 5-7). First-order denitrification potential rate constants had a Pearson correlation of 0.867 with a *p*-value <0.01 level for all sites examined in this study and of 0.875 (*p*-value of 0.002) when compared with just the acid sites (Table 5-3).

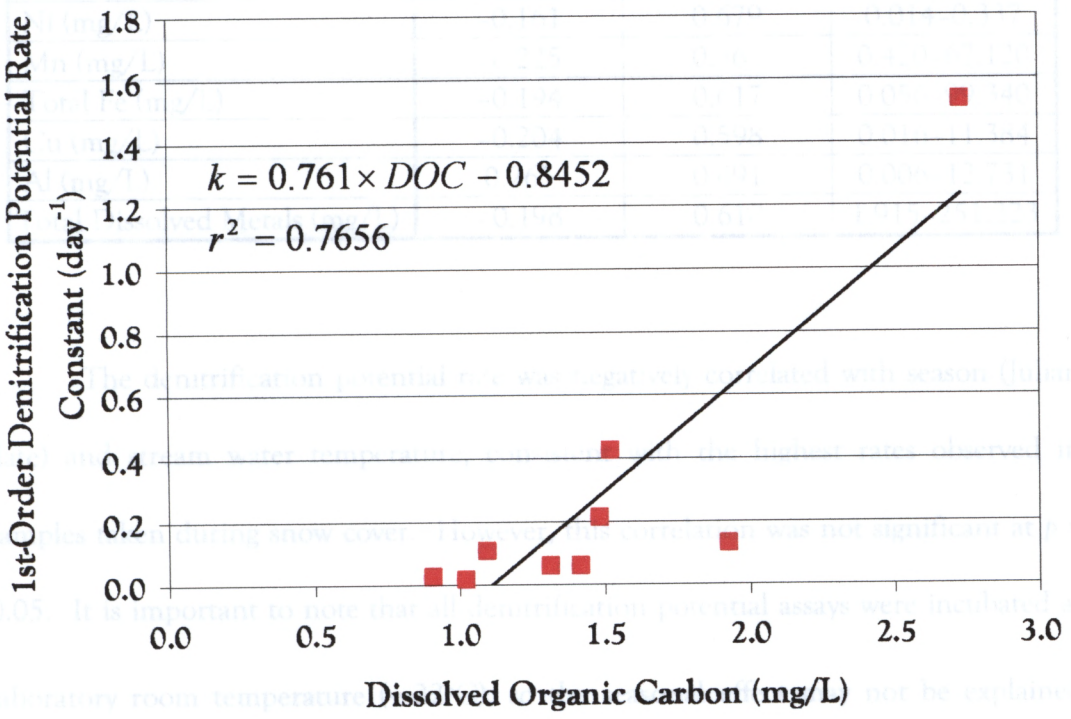

Figure 5-7. First-order denitrification potential rate constants (*k*) vs. DOC concentration in acid-impacted sites near Keystone, Colorado

Table 5-3. Pearson Correlations Between First-Order Denitrification Potential Rate Constants and Various Water Chemistry Parameters in Acid-Impacted Mountain Stream Sediments Near Keystone, CO, during Snow Cover, Snowmelt and Summer Flow

Denitrification Potential Rate Constant Correlated to:	Pearson Correlation Coefficient	*p*-value 2-tailed Significance	Range of Values
Julian Date	-0.572	0.108	112-204
°C of Stream Water	-0.542	0.132	2.2-13.2
pH	-0.263	0.494	3.05-5.64
NO_3-N (mg/L)	0.039	0.920	0.00-0.87
NO_2-N (µg/L)	-0.166	0.670	0.09-0.49
N_2O-N (µg/L)	0.586	0.097	0.04-0.23
NH_4-N (mg/L)	0.645	0.061	0.00-0.10
DOC (mg/L)	**0.875**	**0.002**	**0.91-2.73**
SO_4^{-2} (mg/L)	-0.189	0.625	40.32-1605.81
Cl^- (mg/L)	0.227	0.557	0.00-1.92
Zn (mg/L)	-0.224	0.563	0.266-72.570
Cd (mg/L)	-0.215	0.578	0.002-0.466
Ni (mg/L)	-0.161	0.679	0.014-0.337
Mn (mg/L)	-0.225	0.561	0.420-67.120
Total Fe (mg/L)	-0.194	0.617	0.056-99.340
Cu (mg/L)	-0.204	0.598	0.016-11.384
Al (mg/L)	0.265	0.491	0.006-12.731
Total Dissolved Metals (mg/L)	-0.198	0.610	1.915-251.223

The denitrification potential rate was negatively correlated with season (Julian date) and stream water temperature, consistent with the highest rates observed in samples taken during snow cover. However, this correlation was not significant at $p < 0.05$. It is important to note that all denitrification potential assays were incubated at laboratory room temperature (~22°C), so the seasonal effect may not be explained directly by temperature. Although pH was not significantly correlated with

denitrification potential rate, the correlation coefficient was negative, indicating a trend of decreasing denitrification potential rate with lower pH.

Both nitrous oxide (N_2O) and ammonium (NH_4-N) were positively correlated with denitrification potential rates: $R^2 = 0.586$ and 0.45, with $p = 0.097$ and 0.061, respectively. Nitrous oxide is an intermediate product of denitrification, and its presence in the stream water would be consistent with denitrification activity. Interestingly, there was no correlation between measured nitrate in the stream water and laboratory microcosm specific denitrification potential rates ($R^2 = 0.039$, $p = 0.92$).

Microcosm denitrification potential rates for acidic stream sediments were not correlated with soluble heavy metals, either individually or the aggregate value for total dissolved metals. As with nitrate, iron can be reduced in anaerobic respiration; however, no relation between soluble iron (likely Fe^{2+} at the ambient pH values) and the microcosm denitrification was observed. Ferrous iron may be used as an electron donor by autotrophic denitrifying bacteria, but this relation was not supported by the correlation analysis. There was a strong positive correlation between total dissolved iron concentrations and zinc, cadmium, nickel, manganese and copper concentrations in the acid-impacted streams, indicating a relation between pyrite oxidation and dissolution of heavy metals—with the exception of aluminum, which was not correlated with any other metal in the acid-impacted streams (data not shown).

Several water quality parameters that may relate to denitrification were correlated (Table 5-4). As expected, season and water temperature were strongly correlated ($R^2 = 0.94$, $p < 0.001$), as were sulfate and total dissolved metals, which is

consistent with the reactions of acid mine drainage (pyrite oxidation and metal leaching). Total dissolved metals were negatively correlated with pH, which is also consistent with acid solubilization of metals (R^2 = –0.576, p = 0.105). Nitrous oxide was highest under snow cover, which correlated negatively with temperature and positively with Julian date (season). In addition, N_2O concentration correlated positively with DOC concentration. All the N_2O correlations with water quality parameters were similar to those for laboratory microcosm denitrification potential rates, indicating that N_2O may be a reliable field indicator for denitrification potential.

Table 5-4. Pearson Correlations Between Various Stream Water Chemistry Parameters from Acid-Impacted Sites Near Keystone, CO, During Snow Cover, Snowmelt and Summer Flow Conditions

	Pearson Correlation Coefficient	*p*-value 2-tailed Significance
Season with Temperature	0.941	0.000
Season with N_2O	-0.588	0.096
Temperature with N_2O	-0.658	0.054
pH with NO_2^-	0.734	0.024
pH with Cl^-	-0.702	0.035
pH with Total Metals	-0.576	0.105
NO_2^- with Cl^-	-0.980	0.000
N_2O with DOC	0.587	0.097
N_2O with Al	0.798	0.010
SO_4^{-2} with Total Metals	0.993	0.000

Discussion

Previous research showed that denitrification can occur in acidic, heavy metal–laden sediments contaminated by mining (Chapter 4). Results from this study provide important evidence for a biogeochemical nitrogen cycle in AMD and naturally acidic

streams. Microcosm incubations indicated that denitrifying microorganisms are indigenous to AMD stream sediments. The year-round presence of nitrous oxide, a denitrification product, which was found to correlate positively with sediment denitrification potential rates measured in laboratory microcosms, suggested that denitrification itself may be a continuous process in the stream sediments, even under snow cover. Nitrate concentrations in the snowpack in these areas were often higher than in the stream water, also supporting in situ uptake of nitrate.

An unexpected result was the maximum denitrification potential rates measured in sediment microcosms taken during snow cover, when water temperatures were coldest. It is important to note that the temperature at which the assays were conducted (~22°C) was significantly higher than ambient (averages: 2.9 for -snow cover, 5.25 for snowmelt, 11.5 for summer), which would have increased the observed rates of denitrification in the laboratory. However, field measurements of nitrous oxide, a denitrification product, were also inversely correlated with water temperature in the field, indicating that denitrification during snow cover may be favored by other conditions that outweigh the effects of temperature; for example, oxygen depletion, DOC availability and stagnant flow. In studies examining denitrification in an acidic forest soil, N_2O production increased with increasing temperatures [Wolf and Brumme 2002]. However, in studies examining in situ denitrification in sediments of the Iroquois River in Illinois, rates of nitrate consumption were highest in May and June [Laursen and Seitzinger 2002]. This same study reported that in the Millstone River in Illinois, the highest rates were in March and the lowest in October [Laursen and

Seitzinger 2002]. Leduc and others [Leduc et al. 2002] found that there was no statistical significance between total numbers of bacterial populations in AMD sediments using most potable number (MPN) incubated at ambient and those at 22°C.

Laboratory denitrification potential rates for the pH range 3.05 to 5.64 presented here show no correlation with the stream pH in these sites. These results are consistent with laboratory denitrification incubations from several AMD sites reported earlier, where sediment from a spring at the toe of a tailings pile (pH 2.6) had a higher denitrification potential rate than the stream the spring flowed into, St. Kevin Gulch (pH 4.2) (Chapter 4).

The higher pH during snow cover at the Pennsylvania Mine site could be explained by slower acid-generating microbial activity; as temperatures warm through the season, microbes could become more active and generate more acid, which would in turn explain the pH decrease. Abiotic acid-generating reactions may also increase with warmer temperatures, but studies have shown that the amount of acid generated by microorganisms present is more important than the quantity of acid generated by abiotic reactions (see [Johnson 1998] for review). Peru Creek exhibits its lowest pH during snow cover, which may be explained by less water entering from upstream pristine tributaries. As the area warms and more water enters from the pristine tributaries, an increase in pH occurs. The pH in the naturally acidic Snake River is highest during snowmelt, likely due to dilution by snowmelt. In Deer Creek, the pristine site, pH remains relatively constant.

Denitrification potential rate constants correlated significantly with DOC concentrations in the stream water. Snake River had the highest rate constant and the highest DOC of the acid-impacted sites, indicating that denitrification in these streams is likely dominated by heterotrophic microorganisms. Autotrophic denitrification could be occurring simultaneously. Although iron could be used as an electron donor in these systems because it is abundant, there is no correlation between denitrification potential rates and dissolved iron concentrations at these sites.

Snowmelt in montane environments often brings a flushing of DOC to many stream systems [Boyer et al. 2000]. Because of the high correlation between DOC and denitrification potential rate constants, higher denitrification potential rates during snowmelt are expected; however, the results of this study show highest denitrification potential rate constants and higher DOC concentrations during snow cover, rather than during snowmelt, in most of these sites. There is evidence that DOC concentrations can be highest before the onset of snowmelt [McKnight et al. 1993], which may be important to consider since our snow cover samples were taken just before the onset of snowmelt. The most acidic site, Pennsylvania Mine, does show a higher DOC concentration during snowmelt, which correlates to a higher denitrification potential rate constant. The DOC concentration may be an important factor when determining denitrification potential rates in AMD-effluent sites. DOC concentrations in the snow were higher than in both AMD-impacted sites, suggesting that heterotrophic activity was occurring in these heavy metal-laden acid streams during snow cover. Both Deer Creek and Snake River had the highest DOC

concentrations during snow cover, with concentrations decreasing over the sampling interval, which could be attributed to increasing plant and microbial growth and, in turn, increasing carbon utilization.

The higher DOC concentrations during snow cover provide more organic electron donors for the heterotrophic denitrification process, which correlates with the higher denitrification potential rate constants shown during snow cover. This indicates that the process of heterotrophic denitrification may be more important for the majority of the year, when these sites are snow-covered, and less important during the short summer season, when nitrate concentrations, as well as DOC concentrations, are lowest. The duration of snowpack on these seasonally snow-covered systems has been shown to be important in determining the spatial and temporal variability of nitrogen export from montane systems [Brooks and Williams 1999], which suggests that denitrification in acid-impacted stream systems may also be influenced by the duration of snow cover. A shorter snow cover season may lead to less heterotrophic denitrification; likewise, autotrophic denitrification may become a more important process during the summer season, when there is less organic carbon. However, at present, there is no evidence that autotrophic denitrification is occurring.

When these AMD systems are covered by snow for long portions of the year, denitrification could become the dominant heterotrophic process. AMD-remediation strategies that use heterotrophic bacteria to inhibit autotrophic iron oxidation [Marchand and Silverstein 2002] may need to take nitrate concentrations and the

heterotrophic denitrification process into account when determining the amounts of carbon needed for remediation.

Nitrate was present in all sites sampled, except during snow cover at the Pennsylvania Mine. Although there was no nitrate detected in the Pennsylvania Mine site during snow cover, there was nitrate in the snow, which was likely interacting with the stream water. (The nitrate concentration in the snow sampled in the Pennsylvania Mine was 37μM.) This could suggest that there is nitrate utilization occurring at this site under snowpack. For example, nitrate could be dissimilatory reduced to ammonium, which would explain the lack of nitrate and the presence of ammonium, or it could be denitrified. Denitrification activity and N_2O, an intermediate in the denitrification pathway, were both present at this site, indicating that denitrification is likely the fate of nitrate at this site. Ammonium was only present in these sites in the snow during snow cover. As snow melts into streams, it carries with it ammonium; however, ammonium was not detected in the surface water at these sites, indicating that there is nitrogen-cycling involving ammonium in these AMD streams. This warrants further investigation.

Nitrous oxide concentrations in the stream water correlated with denitrification potential rate constants to a 0.097 significance level, which indicates that as denitrification activity increases, so does the presence of N_2O in the stream water. N_2O concentrations were lowest during summer conditions, which correlates with the lowest denitrification potential rate constants. Higher stream water N_2O concentrations also correlated to higher DOC concentrations, which, in turn, correlates to higher

denitrification potential rate constants. N_2O concentrations correlated with temperature and season. This increase could be explained by a lower solubility of N_2O when stream temperatures increase, or, because nitrous oxide can be produced by nitrification, it is possible that lower nitrous oxide concentrations in the summer resulted from lower availability of ammonium.

Nitrite, an intermediate in denitrification, is highest in the snow and in the pristine Deer Creek, possibly because of the reactivity of nitrite at low pH. A study examining the chemical decomposition of nitrite in sterile acidic soil showed that nitrite was chemically converted to nitric oxide or nitrogen dioxide, with no accumulation of N_2O [Bollag et al. 1973]. In that study, N_2O only appeared as an intermediate in biologically active soils [Bollag et al. 1973]. Thus, because N_2O is present in the water systems tested here, it is likely that nitrite in these systems is biologically converted to N_2O.

Nitrite can be oxidized to NO_2 by free hydroxyl radicals from the photolysis of $FeOH^{2+}$, a form of Fe^{3+} [Zhang and Bartlett 2000]. This may be an important fate of nitrite in AMD systems, particularly during the daytime hours when hydrous iron oxides and dissolved Fe^{3+} are subject to photoreduction by sunlight [McKnight et al. 2001]. The abiotic nitrite reactions may also be important in the cycling of organic matter in these low pH streams. NO_2^- can be a strong scavenger, competing with organic compounds for hydroxyl radicals [Zhang and Bartlett 2000]. DOC concentration and composition are also affected where iron oxyhydroxides are present, such as in AMD- or ARD-impacted streams [McKnight et al. 2002]. The interaction of

nitrite, DOC and iron compounds, which may be important in AMD streams, may be seasonally influenced by DOC flushing, nitrate pulses in snowmelt, increased nitrite production from denitrification or other processes. This topic warrants further investigation.

Sulfate concentrations in the pristine stream remained low throughout the season, which is expected because the stream was not impacted by acid rock or acid mine drainage [Boyer et al. 2000]. In the acidic streams, the sulfate concentration tended to be inversely related to the pH and positively correlated to total dissolved metal concentrations, which is indicative of acid rock and acid mine drainage. During snowmelt, sulfate concentrations decreased at all sites, indicating that dilution from snowmelt would likely play an important role. Sulfate, however, had no correlation to denitrification potential rate constants, indicating that it may not influence denitrification activity in acid-impacted stream systems.

Denitrification potential rate constants correlated neither to any specific metal nor to total dissolved metal concentrations, which may indicate that denitrifying activity in AMD streams is independent of metals and, thus, may not be an important consideration in these sites. Many metals found in these streams are often considered toxic at low concentrations. For example, the EPA recommends a surface water quality standard for zinc of less than 0.12 mg/L to maintain aquatic life [E.P.A. 1999]. Zinc concentrations in all three of the acid-impacted streams examined here exceeded that recommendation. Because zinc does not correlate to denitrification potential rate

constants, however, it does not seem to have a direct effect on the microbial denitrification activity in these environments.

Metal concentrations were highest in the Pennsylvania Mine site, which would be expected because it is direct mine runoff. Most metal concentrations were lowest during snowmelt, showing a dilution effect, which may require further examination because previous research has shown that metal concentrations are often higher during snowmelt because of a flushing effect [August et al. 2002, Brooks et al. 2001]. In the Pennsylvania Mine site, concentrations of metals were highest during the summer months, perhaps because of higher microbial activity, which corresponds to the lower pH and higher sulfate concentrations.

Conclusions

Until now, nitrogen cycling in acid-impacted streams has been neglected. Results from this research show that nitrogen species are present in AMD streams throughout the year, indicating that there is biogeochemical nitrogen cycling in these low-pH, heavy metal–laden streams. Microbes capable of denitrification are present and active during the entire year and are perhaps most active during the majority of the year when these montane regions are covered in snow. Denitrification potential rate constants did not correlate with pH or dissolved metals, indicating that the contamination effects of low pH and high heavy metal concentrations in AMD-impacted streams does not control denitrification activity. Denitrification is most influenced by the amount of DOC present in the surrounding waters, indicating that

heterotrophic denitrification may be an important means of nitrate removal from these streams. This process is important to consider when examining nutrient budgets and nitrogen saturation of montane ecosystems due to increasing nitrogen deposition.

Results from this work show that heterotrophic denitrification needs to be considered when determining remediation strategies for AMD-impacted sites. Denitrification could be a competitive process to heterotrophic iron metabolism, or, because heterotrophic denitrification produces alkalinity, it could increase remediation efforts.

CHAPTER 6:

Microbial Diversity of Acid-Impacted Mountain Stream Sediments during Snow Cover, Snowmelt and Summer

Abstract

Nitrogen deposition is increasing in the Rocky Mountains of Colorado, raising concern for the ability of the microbial community to metabolize excess nitrate entering streams via atmospheric deposition. Many of these streams are also contaminated by acid mine drainage. Little is known about the ability of microbial communities in acidified streams to cycle nitrogen. Microbial diversity studies on AMD have mainly focused on acid generation (iron, sulfur and metal cycling by microorganisms living in waste rock, or tailings, and mine drainage water), rather than on nitrogen cycling. In this research, the microbial communities in sediments collected from two AMD-impacted streams, a naturally acidic stream and a pristine circumneutral stream were surveyed during snow cover, snowmelt and summer conditions by analyzing clone libraries of rRNA genes amplified from environmental DNA. More than 1,340 individual clones were analyzed, including members from all three domains, as well as from 21 different bacterial divisions. Overall, the communities were dominated by proteobacteria, with the exception of Pennsylvania Mine effluent and Peru Creek and Snake River sediments during the snow-cover season. The total clones from Peru Creek and Snake River contained high percentages of Acidobacteria. Deer Creek had more clones belonging to the Acidobacteria division

than did Pennsylvania Mine, the most acidic site examined. Interestingly, no bacteria clones matched 16S rRNA sequences of strains commonly identified as denitrifiers in soils and sewage, namely, *Pseudomonas denitrificans, Ps. stuzeri, Ps. aeruginosa* and *Paracoccus denitrificans.*

Introduction

Increased nitrogen deposition in the Colorado Rocky Mountains has resulted in elevated nitrate concentrations in many stream systems [Williams 2000]. Streams originating in this area provide much of the drinking and irrigation water for the intermediate west and the Great Plains. Of increasing concern is the ability of mountain ecosystems to process the excess nitrogen loads [Brooks and Williams 1999, Williams 2000]. Biological denitrification, the reduction of nitrate to nitrogen gas, is the primary permanent biological removal process of nitrate from water and soil. Facultative heterotrophic bacteria under anoxic conditions, where nitrate is the electron acceptor for respiration, carry out most environmental denitrification [Zumft 1997].

Many of the streams in the western United States are also affected by acid mine drainage. The U.S. Forest Service reports that 8,000–16,000 km of streams in the forest service land of the western United States are affected by AMD [USFS 1993]. Many abandoned mines from years of mining activity during the mid-1800s to the early 1900s can be found throughout Colorado. Tailings piles, or waste rock, from these mines dot the Colorado landscape. Weathering of pyrite minerals (FeS_2) in the

tailings generates iron and sulfuric acid [EPA 1994], which solubilizes heavy metals, such as Zn, Al, Pb and Cd, in minerals also present in the rocks. The presence of microorganisms, such as *Acidithiobacillus ferrooxidans* and *Leptospirillum ferriphilium*, increase the rate of pyrite dissolution as well as the amount of acidic, heavy metal-laden leachate from the tailings piles [Baker and Banfield 2003]. This leachate runs off into nearby streams, creating an extreme environment for life. Streams can also be affected by the more general acid rock drainage (ARD), caused by the weathering of naturally occurring pyritic minerals in the bedrock of stream basins. however, ARD occurs to a much lesser extent than does AMD.

AMD and ARD environments can provide many challenges for microbial life [Madigan 2000], and much research has been done to characterize the microbial community responsible for acid generation in these systems (see [Baker and Banfield 2003] for review). Recently, these AMD communities have been studied using nonculturing methods, such as 16S rRNA, to characterize microbial communities and has focused on the tailings and mine sites (e.g., [Bond, et al. 2000, Edwards, et al. 1999, Leduc, et al. 2002]). However, relatively little work has been done to characterize the downstream effects of AMD and, to a lesser extent, ARD on microbial communities.

The impact of AMD on the biogeochemical nitrogen cycle in mountain streams is unknown. Until recently, denitrification in acid-impacted stream environments was thought not to occur (see Chapters 4 and 5). Microcosm studies showed that although nitrate in these streams can be reduced completely to nitrogen gas without the addition

of electron donors, increased amounts of carbon stimulated the denitrification potential rates. Heavy metals and pH seem to have little effect on the process. To this point, it is unclear which microorganisms are capable of acidic denitrification.

Denitrifiers typically make up 0.1-5% of the total culturable population in mineral soils [Tiedje 1994]. Denitrification activity has been reported in more than 50 genera and almost 130 species and is considered a widely diverse microbial metabolism [Tiedje, et al. 1989, Zumft 1992]. However, denitrifiers frequently encountered in soil are from limited phylogenetic groups, with more than 50% of the dominant soil denitrifiers belonging to the genus *Pseudomonas*, a group of gamma proteobacteria [Tiedje 1994]. Other important genera of denitrifiers are *Alcaligenes*, *Bacillus*, *Agrobacterium* and *Flavobacterium*. Denitrifiers have also been found under select conditions in *Rhizobium*, *Bradyrhizobium*, *Axospirillium*, *Nitrosomonas*, *Nitrobacter* and *Thiobacillus*, and occasionally in *Paracoccus*, *Cytophaga*, *Flexibacter*, *Chromobacterium* and *Gluconobacter* [Tiedje 1994].

Given increasing anthropogenic sources of nitrogen into AMD-influenced watersheds, the capacity of acidic, heavy metal-laden streams to assimilate nitrogen, mainly in the form of nitrate, is of interest. The objective of this study was to use culture-independent methods to characterize the microbial population in acidified streams by examining small-subunit (SSU) rRNA genes amplified from the sediment community DNA. Of particular interest was identification of organisms known to denitrify.

Materials and Methods

Site Description

Four mountain streams with varying degrees of acid impact near Keystone, Colorado, were chosen for this study. A small drainage stream flowing from the abandoned Pennsylvania Mine was chosen to assess the microbial diversity in a direct effluent AMD site. This effluent stream drains into Peru Creek, a higher-order stream affected by several AMD tributaries. Peru Creek, which eventually flows into Snake River, was selected to examine downstream effects of AMD on the microbial community structure. Snake River and Deer Creek are located in a basin adjacent to the AMD-impacted sites. The upper reach of the Snake River is naturally acidic because of the pyritic bedrock in the drainage basin. Downstream from the sampling site, drainage from abandoned mines affects the water quality of this tributary to Dillon Reservoir, a major water supply for the Denver metropolitan area. The upper Snake River was selected to examine differences in a naturally acidic stream environment as opposed to anthropogenic AMD-impacted sediments. Deer Creek, a pristine stream that converges with Snake River just downstream of the sampling site, was sampled to examine possible diversity differences between a pristine mountain stream sediment and nearby acid-impacted streams.

Sediment Sample Collection

Triplicate sediment samples (0–3cm depth) were collected during snow cover (April 22, 2003), snowmelt (June 6, 2003) and summer (July 21, 2003) from each of the

acid-impacted mountain streams, as well as from the pristine site. The samples were placed in 15mL sterile tubes and were frozen until analysis.

Surface Water Sample Collection and Analysis

Stream water samples and snow, when present, were collected from each site and taken to the lab for immediate preservation and analysis. pH was measured immediately with an Orion pH probe. Samples for anion determination were filtered through a 0.1 μm Millipore filter, preserved by freezing and analyzed using a Dionex 500 Ion Chromatograph with carbonate- buffered eluent (1.8 mM Na_2CO_3/ 1.7 mM $NaHCO_3$, pH 7.5) at a flow rate of 2.00 mL/min through an IonPac AS4A analytical column with an AG4A guard column and a conductivity detector. Samples for ammonium determination were filtered through a 0.1 μm Millipore filter, preserved with concentrated sulfuric acid to a final pH of 2 and analyzed using a Dionex 300 Ion Chromatograph with a gradient elution of 2.5 to 25mN sulfuric acid at 1.5 mL/min through a CS12A analytical column and CG12A guard column and a conductivity detector. Samples for nitrite analysis were filtered through a 0.1 μm Millipore filter and frozen until analysis. Nitrite was reduced to nitric oxide with 0.5 mL of 2% sodium iodide added to 4mL of glacial acetic acid, exposed to ozone and passed through a chemiluminescent nitric oxide detector (Sievers Analytical, Model NOA 280, Boulder, CO). Nitrous oxide samples were collected in the field in 38 mL serum bottles prepared with 200 μL of 12.5 N NaOH, sealed with thick butyl stoppers, crimped, flushed with helium (for 15 minutes) and evacuated (to 15 psi for 5 minutes).

In the field, triplicate samples were taken by adding 15 mL of surface water with a syringe into each serum bottle. Nitrous oxide in the headspace was measured using a gas chromatograph (GC Model 301, HNU Systems) equipped with a wide-range electron-capture detector (Model 140 BN, Valco Instruments Co., Inc.) using 5% methane/95% argon carrier gas; the instrument was calibrated with standard gas mixtures (Scott Specialty Gases, Plumsteadville, PA). DOC samples were collected in the field in ashed amber bottles and filtered through ashed GF/F filters before storing at 4°C until analysis using a Total Organic Carbon Analyzer (Shimadzu Corp. TOC-5050, Kyoto) within three weeks of sample collection. For dissolved trace metals, stream water was filtered through 0.1 μm Millipore filters, preserved with trace metal-grade hydrochloric acid and analyzed by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) (Liberty Series 2, Varian Corporation).

Environmental Genomic DNA Extraction

Total environmental community genomic DNA was extracted using a solvent and bead-beating extraction protocol, slightly modified from a previously described method [Dojka, et al. 1998], from each triplicate sediment sample, which were pooled to reduce spatial variation in the stream sediment environment. Briefly, DNA was extracted from ~0.5 g of sediment with 0.7 mL acid extraction buffer (400 mM TRIS, pH 8.0, 100 mM EDTA, 6% (wt/vol) SDS), 0.5 mL buffer saturated (pH 7.5) phenol/chloroform/isoamyl-alcohol (24:24:1), and 0.5 g of 0.1mm zircon/silica beads

(BioSpec Products Incorporated). All procedures were performed in a UV-sterilized AirClean 600 PCR workstation (AirClaen Systems).

PCR Amplification and rDNA Clone Libraries

Community small subunit (SSU) ribosomal RNA (rRNA) genes (rDNA) were amplified by polymerase chain reaction (PCR) using extracted genomic DNA as template and universal primers (below). Each 20 µl PCR reaction contained ~50 ng of extracted DNA template, 1X HotMaster Buffer (Eppendorf), 200 µg/ml Bovine Serum Albumin (BSA), 50 µM (each) deoxynucleotide triphosphates, 0.2 µM each of forward and reverse primer and 1.25 U of HotMaster Taq DNA Polymerase (Eppendorf). PCR reaction mixtures were incubated in a MasterCycler programmable thermal cycler (Eppendorf) at 94°C for 2 minutes (for initial denaturation), followed by 30 cycles of 94°C for 20 seconds, 52°C for 20 seconds and 65°C for 90 seconds, followed by a final extension period of 10 minutes at 65°C. The primers used in this study were 515F (universal) (5′-GTGCCAGCMGCCGCGGTAA-3′) and 1391R (universal) (5′-GACGGGCGGTGWGTRCA-3′).

Environmental rDNA clone libraries (collection of randomly selected clones) were constructed from PCR-amplified products purified by gel electrophoresis using the Montage DNA Gel Extraction Kit (Millipore) and cloned using the TOPO TA Cloning Kit (Invitrogen), as specified by the manufacturers. Plasmid DNAs containing inserts were screened by restriction fragment length polymorphism (RFLP) analysis [Dojka, et al. 1998] using Msp I and HinP1 I restriction endonucleases (New England

Biolabs). PCR products from rDNA clones with unique RFLP patterns were prepared for DNA sequencing using the ExoSAP-it for PCR Cleanup Kit (USB) and sequenced with twofold coverage (vector primers T3 and T7) using a MegaBACE 1000 DNA sequencer (Amersham Biosciences) and DYEnamic ET Terminator Cycle Sequencing Kit reaction mixtures (Amersham Biosciences).

In total, we constructed 12 libraries, each with either 96 or 192 (see below) randomly selected clones, from individual PCR reactions performed with the universal primer pair 515F and 1391R on each DNA sample. PCR reactions were performed for each replicate DNA and cloned separately to yield 12 universal libraries. PCR reactions of 5 of the 12 DNA samples (Pennsylvania Mine snow cover, Deer Creek summer and all Snake River samples) showed two distinct bands during gel purification. When present, these bands were purified and analyzed separately. Because no strong differences were noted between the bands, the sequences from the two bands were combined and treated at one library in all further analyses.

Phylogenetic Analysis

DNA sequence analysis, assembly, initial characterization and databasing were performed with the XplorSeq software package [Frank 2003-2004]. Within XplorSeq, raw DNA sequencer trace data was analyzed with the PHRED software package [Ewing and Green 1998, Ewing, et al. 1998] to assign bases and quality scores. Partial sequences were assembled into contiguous sequences using the PHRAP software package [Ewing and Green 1998, Ewing, et al. 1998]. rDNA sequences were initially

compared with a current database of genetic sequences (GenBank) using the BLAST (Basic Local Alignment Search Tool) network service [Altschul, et al. 1997] to determine their approximate phylogenetic affiliation. Complete rDNA sequences were exported from XplorSeq and aligned to other known SSU-rRNA sequences using the ARB software package [Strunk, et al. 1998]. Sequence alignments used to infer phylogenetic relationships were created using the Lane mask [Lane, et al. 1985], which excludes hypervariable regions of the SSU-rRNA sequence. Sequence information from the sites with two bands showed little difference and, thus, were pooled together by site and sampling time.

Statistical Analysis

Pearson correlations were computed to examine relationships between denitrification potential rate constants (from Chapter 5) and bacterial divisions using the SPSS Database Software Program for Windows 10.0.5 [SPSS 1999].

Accession Numbers

The sequences of all the rDNA clones analyzed will be entered into GenBank.

Results

Results from water chemistry analysis are presented in Table 6-1. (See Chapter 5 for a more complete analysis.) Background water chemistry from the acid-impacted sites showed a pH range between 3.05 and 5.64, with no distinct seasonal pattern.

Temperature corresponded with season. DOC concentrations were low at all sites, with the highest concentrations in the pristine Deer Creek site. Sulfate concentrations were highest in the Pennsylvania Mine effluent sites and lowest in the pristine Deer Creek. Nitrate concentrations were lowest during the summer in all sites sampled. Nitrate was present at each site during all three seasons, except in Pennsylvania Mine during snow cover. Ammonium was only present during snow cover in all sites, except for Peru Creek, where no ammonium was detected. Dissolved iron and aluminum concentrations were highest in the mine effluent stream. Zinc concentrations exceeded the EPA's water quality standard of 0.12 mg/L to maintain aquatic life [U.S.E.P.A. 1999] in all sites except the pristine Deer Creek.

The microbial communities of three sediments with varying degrees of acidic influences and a pristine sediment had different relative percentages of microorganisms that vary seasonally (Figure 6-1). The organisms in these communities were predominately Bacterial, with Eucaryotes and Archaea not always present. When present, Eucaryotes, mainly algal species, dominated during snow cover, making up 34% and 37% of the sequences analyzed in sediment from Pennsylvania Mine effluent and from Snake River, respectively. Eucaryotes were only detected in Deer Creek in summer (4%) and not detected during any season in Peru Creek. Archaea, considered important and abundant in some AMD environments [Baker and Banfield 2003], were not detected in either of the AMD-impacted sites. Molecular phylogenetic analysis of 1,345 rDNA sequences showed that the community comprised organisms with rDNA

sequences representative of ~40% (21/52) of the major identifiable bacterial divisions (Table 6-2).

Table 6-1. Water Chemistry Parameters from Acid-Impacted Mountain Streams During Snow Cover, Snowmelt and Summer 2003

	Pennsylvania Mine			Peru Creek			Snake River			Deer Creek		
	Mine Effluent			*AMD-Impacted*			*Naturally Acidic*			*Pristine*		
	Snow cover	Snow melt	Summer	Snow cover	Snow melt	Summer	Snow cover	Snow melt	Summer	Snow cover	Snow melt	Summer
Denitrification potential rate Constants (day^{-1})*	0.11	0.15	0.06	0.43	0.02	0.03	1.54	0.09	0.02	1.51	0.94	0.66
Temp (°C)	3.6	5.3	12.1	3.7	5.4	13.2	2.2	5.1	10.4	2.7	5.1	10.2
pH	4.67	4.16	3.05	3.74	5.15	5.64	3.90	4.30	3.99	6.21	6.34	6.36
DOC (mg/L)	1.06	1.48	1.31	1.52	0.91	1.42	2.73	1.93	1.02	4.01	3.65	1.94
SO_4^{2-} (mg/L)	733	314	1610	103	42.2	45.8	130	40.3	65.6	24.1	4.2	11.3
NO_3-N (mg/L)	0.00	0.87	0.46	0.59	0.64	0.56	0.49	0.48	0.47	0.54	0.51	0.45
NH_4-N (mg/L)	0.10	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.02	0.00	0.00
Fe_{Total} (mg/L)	14.5	5.11	99.3	0.26	0.42	0.06	0.96	0.53	0.79	0.14	0.05	0.06
Al (mg/L)	12.1	12.7	56.8	2.50	0.42	0.08	7.40	1.90	3.37	0.01	0.01	0.01
Zn (mg/L)	24.3	13.5	72.6	2.16	0.70	0.85	1.00	0.27	0.55	0.08	0.02	0.02

* Denitrification potential rate constants were determined in previous experiments (See Chapter 5)

Table 6-2. Number of Established Bacterial Divisions Present in Acid-Impacted Mountain Stream Sediment Samples Detected by 16S rRNA

	Pristine	Acid Impacted	AMD	Naturally Acidic
Snow cover (4/22/03)	13	8	8	6
Snowmelt (6/05/03)	11	11	10	9
Summer (7/23/03)	15	15	10	9
Total Divisions	**18**	**17**	**16**	**11**

21 divisions present in all samples analyzed.

* Acid-impacted streams include all three acidic streams: Pennsylvania Mine effluent, Peru Creek and the naturally acidic Snake River. AMD-impacted streams include the Pennsylvania Mine and Peru Creek.

The results show no consistent trend in community bacterial divisional diversity among snow cover, snowmelt and summer conditions. In the AMD-impacted sites, the percentage of the population consisting of Proteobacteria increased from snow cover to snowmelt. Peru Creek sediments were dominated by Acidobacteria during snow cover, with the percentage decreasing throughout the seasons. Actinobacteria were generally highest under snow cover. In the naturally acidic Snake River site, the bacterial diversity percentages stayed relatively constant, with the exception of increases in Chloroflexi during snowmelt and Actinobacteria during snowcover.

From the clone libraries and RFLP analysis, 843 unique clones were sequenced and imported into ARB for phylogenetic analysis. Cluster analysis showed that these sequences belong to 395 distinct relatedness groups with ≥97% sequence identity, which corresponds approximately to the rRNA sequence variation in microorganisms at the species level [Stackebrandt and Goebel 1994], and to 313 relatedness groups with ≥95% sequence identity, which corresponds approximately to the genus level [Stackebrandt and Goebel 1994]. A comparison of the sequences from this study to the >200,000 rRNA sequences in the public database, GenBank, showed that the average sequence identity from these mountain stream sediments, as compared with known species, was 96% (Table 6-3). Approximately 52% of the mountain stream sediment 16S rRNA sequences were <97% identical (species level) to known sequences in GenBank, and ~24% of the sequences are <95% identical (genus level) to sequences in GenBank.

Table 6-3. Summary of 16S rRNA BLAST Database Identification by Percent

	Pennsylvania Mine			Peru Creek			Snake River			Deer Creek		
	Mine Effluent			*AMD- Impacted*			*Naturally Acidic*			*Pristine*		
	Snow cover	Snow melt	Summer	Snow cover	Snow melt	Summer	Snow cover	Snow melt	Summer	Snow cover	Snow melt	Summer
Average % ID from BLAST	97.1	95.5	95.6	96.6	95.6	95.6	96.1	95.2	95.5	95.4	94.3	95.6
<97% ID	22.6	36.7	34.1	71.6	56.9	56.4	64.2	66.4	52.6	62.8	76.3	24.6
<95% ID	10.4	23.3	9.8	4.5	26.2	28.2	10.8	31.8	29.5	43.6	50.0	15.0
Number of Clones	115	90	82	88	65	78	177	173	156	78	76	167
No. of Unique Clones	75	46	38	28	65	78	41	76	79	78	76	167

The bacterial community in these sites was mainly dominated by Proteobacteria (~8-~79%). *Alpha-*, *Beta-*, *Gamma-* and *Delta-Proteobacteria* subdivisions were present in all samples, with varying percentages (Table 6-4). Only one *Acidiphilium* clone, a common AMD bacteria [Baker and Banfield 2003], was identified out of the 1,345 clones analyzed. Of the sequences in this study, 5.4% fell into a novel relatedness group within *Burkholderiales*, termed "*Burkholderiales*, ARD clones" for this analysis. Within the *Gamma-Proteobacteria*, 2.9% of the sequences fell into a related grouping within the Environmental Clone Group, which was termed "Environmental Clone Group, ARD clones" for this analysis. *Xanthomonadales* made up 3.8% of the total group of sequences, with 1.5% of the total sequences belonging to a group with sequences from GenBank found in AMD environments and 2.3% assembled into a separate relatedness group from mainly the Pennsylvania Mine and Peru Creek sites, termed "*Xanthomonadales*, ARD clones." The two subgroups within *Xanthomonadales* are closely related to an Fe^{2+}-oxidizing denitrifying clone identified by Straub and Buchholz-Cleven [Straub and Buchholz-Cleven 1998].

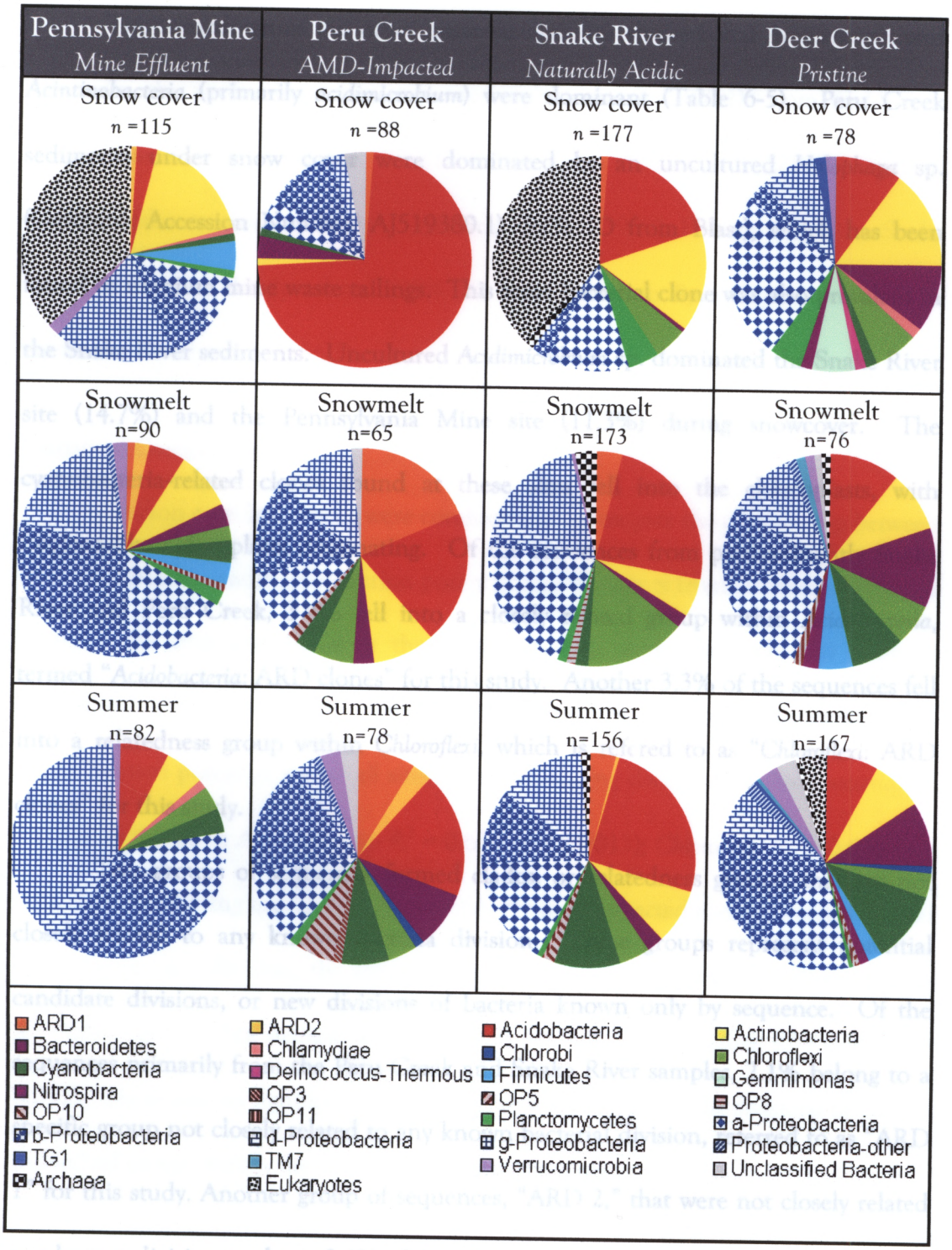

Figure 6-1. 16S rRNA diversity from acid-impacted mountain stream sediments during snow cover (4/22/03), snowmelt (6/5/03) and summer (7/23/03). Each pie diagram represents three sediment samples (top 3cm), from which genomic DNA was extracted and pooled to reduce possible spatial variation effects.
***n* = number of clones sequenced from each sample**

The breakdown of the other bacterial divisions showed that *Acidobacteria* and *Acintinobacteria* (primarily *acidimicrobium*) were dominant (Table 6-5). Peru Creek sediments under snow cover were dominated by an uncultured *Holophaga* sp. (GenBank Accession Number: AJ519380.1) (97% ID from Blast), which has been found in uranium mine waste tailings. This same bacterial clone was also prevalent in the Snake River sediments. Uncultured *Acidimicrobium* sp. dominated the Snake River site (14.7%) and the Pennsylvania Mine site (11.3%) during snowcover. The cyanobacteria-related clones found at these sites fell into the chloroplasts, with Euglena-like chloroplasts dominating. Of the sequences from predominantly Snake River and Peru Creek, 3.4% fell into a closely related group within *Acidobacteria*, termed "*Acidobacteria*: ARD clones" for this study. Another 3.3% of the sequences fell into a relatedness group within *Chloroflexi*, which is refered to as "*Chloroflexi*: ARD clones" for this study.

Two groups of sequences formed clades, or relatedness groups, that are not closely related to any known bacteria division. These groups represent potential candidate divisions, or new divisions of bacteria known only by sequence. Of the sequences primarily from the Peru Creek and Snake River samples, 2.1% belong to a specific group not closely related to any known bacterial division, referred to as "ARD 1" for this study. Another group of sequences, "ARD 2," that were not closely related to a known division made up 0.4% of the sequences.

Archaea were only present in the naturally acidic and pristine sites at 1.8% and 0.6%, respectively, of the total clones from each site (Table 6-5). Eucaryotes, when

present, were dominated by *Chrysophytes*, or golden-brown algae. In the Pennsylvania Mine site, the predominant phylotype was a close match (98% ID from BLAST) to *Chrysolepidomonas dendrolepidata* (GenBank Accession Number: AF123297) isolated from a freshwater lake with pH 7 [Andersen, et al. 1999]. Eucaryotes in the Snake River were most closely related (95% ID from Blast) to *Synura spinosa* (GenBank Accession Number: M87336.1).

Statistical Comparison

Pearson correlation tests were conducted to investigate the relationship between bacterial divisions and denitrification potential rate constants from sediments collected at the same sites and times as those used in this study for molecular analysis. Significant correlations were found between 26.5% of the total clones and denitrification potential rate at all sites for the following divisions—α-*Proteobacteria:* $R^2 = 0.860$ ($p < 0.001$); *Actinobacteria:* $R^2 = 0.690$ ($p = 0.0013$); *Chloroflexi*: $R^2 = 0.638$ ($p = 0.025$). In examining the correlation for only the acid-impacted sites between rates and clones from each division, the significant correlations are as follows—α-*Proteobacteria:* $R^2 = 0.885$ ($p = 0.002$); *Actinobacteria:* $R^2 = 0.717$ ($p = 0.03$); *Chloroflexi:* $R^2 = 0.709$ ($p = 0.032$). There was no significant correlation between AMD sites to divisions or among individual groups within the divisions and rate constants.

Table 6-4. Percentage of Total 16S rRNA Clones from Sediments of Two AMD-Impacted Mountain Streams, a Naturally Acidic Stream and a Pristine Mountain Stream Divided into Various *Proteobacteria* Subdivisions

(SC= snow cover, SM= snowmelt, S= summer)

	Pennsylvania Mine			Peru Creek			Snake River			Deer Creek		
	Direct Mine Effluent			*AMD-Impacted*			*Naturally Acidic*			*Pristine*		
	SC	SM	S	SC	SM	S	SC	SM	S	SC	SM	S
α-Proteobacteria	**7.0**	**15.5**	**13.4**	**4.5**	**1.8**	**1.3**	**13.0**	**19.8**	**16.7**	**19.2**	**15.8**	**14.4**
Acetobacteraceae, environmental	7.0	3.3	8.5		1.5	2.6		2.3	3.3		1.3	0.6
Acetobacteraceae, *Acidiphilium*		1.1										
Bradyrhizobiaceae, *Bradyrhizobium*				2.3	1.5	2.6	9.6					0.6
DA122 soil group					1.5	1.3				2.6		
Environmental		11.1	3.7	2.3	6.2	3.8	3.4	16.8	9.6	7.7	1.5	8.4
Hyphomicrobiaceae2										1.3		
Rhickettsiales			1.2									
Rhizobiales										2.6		
Rhodobacterales									3.8	1.3	2.6	3.0
Sphingomonadales										1.9	1.3	1.8
β-Proteobacteria	**7.0**	**3.0**	**23.3**	**1.2**	**13.8**	**23.3**	**1.7**	**11.6**	**11.5**	**25.6**	**11.8**	**18.6**
Burkholderiales, environmental									4.5	5.1		1.8
Burkholderiales, *Burkholderiaceae*	0.9									1.3		
Burkholderiales, *Comamonadaceae*				2.3		2.6	1.1	2.3	0.6	11.5	1.3	4.2
Burkholderiales, *Oxalobacteraceae*										1.3		0.6
Burkholderiales, *ARD clones*[1]	4.3	3.0	17.7	3.5	3.8	6.4	0.6	9.2	5.1		5.3	5.4
Environmental	1.7		7.0	3.5	1.8	6.4			1.3	6.4	3.9	5.4
Methylophilus et.al				1.1							1.3	1.2
δ-Proteobacteria		**2.2**	**4.9**		**4.6**	**1.3**		**2.9**	**2.6**	**2.6**	**5.3**	**4.2**
Bdellovibrionales					1.5							
Desulfuromonadales, *Geobacteraceae*										1.3	3.9	1.8
Desulfuromonadales, environmental		2.2	4.9		1.5						1.3	1.2
Environmental					1.5			2.3	1.3			
Myxococcales						1.3		0.6	1.3	1.3		1.2
γ-Proteobacteria	**16.5**	**15.6**	**35.4**	**2.3**	**7.7**	**12.8**	**0.6**	**13.3**	**1.3**	**3.8**	**5.3**	**3.0**
Environmental						1.3					1.3	0.6
Env. Clone Group, environmental					1.5					3.8	1.3	0.6
Env. Clone Group, ARD clones[1]			3.7		6.2	1.3	0.6	8.7	9.6			
γ- Proteobacteria A	2.7										2.6	1.8
Xanthomonadales, ARD clones[1]	12.2	14.4	22.0	2.3					0.6			
Xanthomonadales, AMD clones	1.7	1.1	9.8			1.3		4.5				
Other *Proteobacteria*		**1.1**				**1.3**					**1.3**	**1.8**
Total Sequences	115	90	82	88	65	78	177	173	156	78	76	167

[1]Clone groups from this study

Table 6-5. Number of 16S rRNA Clones from Two AMD-Impacted Mountain Stream Sediments Compared with a Naturally Acidic and a Pristine Mountain Stream in Major Groupings

ARD 1 and ARD 2 are distinct groups of sequences from this study that did not fall into an established bacterial division.

***n* = clone group (SC = snow cover, SM= snowmelt, S= summer)**

	Pennsylvania Mine			Peru Creek			Snake River			Deer Creek		
	Direct Mine Effluent			*AMD-Impacted*			*Naturally Acidic*			*Pristine*		
	SC	SM	S	SC	SM	S	SC	SM	S	SC	SM	S
ARD 1[1]		1		1	12	6	2	6	6			
ARD 2[1]	1	2				2			1			
Acidobacteria	3	5	6	64	13	13	33	37	39	6	6	13
Acidobacteria-1	3	4	4	6	1	7	12	16	18		2	2
Acidobacteria-3		1		52	4	1		3	6		1	3
Acidobacteria-4										3		1
Acidobacteria-6										3	4	6
ARD clones[1]				5	8	3	9	10	11			
environmental			2	1		2	12	8	4		1	1
Actinobacteria	18	8	5	1	6		27	9	13	9	7	13
Acidimicrobidea, acidimicrobium	13	1	4		6		26	8	8	6	4	6
other	5	7	1	1			1	1	5	3	3	7
Bacteriodetes		3		3	2	6	1	3	4	6	9	16
Saprospiracea				1	1	3		3	2	6	6	11
other		3		2	1	3	1		2		3	5
Chloroflexi		2	3		4	3	9	24	7	4	5	6
ARD clones[1]		2	3		3	3	9	13	7		2	2
Chloroflexi 1					1			11		4	3	4
Cyanobacteria	10	3	3	1	2	5		3	16	1	6	18
Firmicutes	5	3									4	4
Alicyclobacillaceae		2										
Bacilli	5	1										
Butycivibrio											1	1
Chlostridium, Calamator											3	3
Planctomycetes	1	2				1	8	2	1	3		1
Proteobacteria	35	58	63	15	24	32	27	81	64	40	30	70
Verrucomicrobia	2	2	1			2		1		1	1	5
Archaea							2	5	2		1	1
Eucaryotes	39						69					7
Apicomplexa							2					1
Environmental	2						14					3
Heterokonta	37						52					3
Total Sequences	115	90	82	88	65	78	177	173	156	78	76	167

[1] Clone groups from this study

Discussion

Of the 52 established divisions of bacteria (to date), 21 are represented in samples collected for this study. This is relatively high diversity compared with other environments, such as a Wisconsin agricultural soil, where 17 divisions were represented [Borneman, et al. 1999], and grass pasture rhizospheres, where 22 divisions were present [McCaig, et al. 1999]. In Iron Mountain, California, perhaps the most well-known and well-studied AMD site, only four divisions of bacteria were found [Bond, et al. 2000]. A recent review article compiled all studies of AMD sites as of 2003 and concluded that 11 divisions were represented [Baker and Banfield 2003]. Results from the Pennsylvania Mine and Peru Creek show 16 divisions represented throughout the year, which includes members from 5 bacteria divisions not previously uncovered in AMD environments. In general, snow cover samples contained the lowest number of divisions, with divisional diversity increasing during snowmelt, possibly due to organisms brought in through runoff through soil vegetation. The highest number of divisions was present during summer, when temperatures were warmer and pHs generally higher, which may be more conducive for colonization and growth by members of other divisions.

A decrease in divisional diversity in Pennsylvania Mine effluent corresponds to decreasing pH and an increase in proteobacteria. This trend was repeated in Peru Creek sediment during snow cover, when the pH, as well as the number of divisions, was lowest. Unlike Pennsylvania Mine, the *Proteobacteria* were not dominant during this period in Peru Creek sediment. It is not clear why Peru Creek did not have a

dominance of *Proteobacteria.* It may be that hydrology is an important factor controlling the microbial diversity of these environments. During snow cover, for example, the Peru Creek drainage would have come mainly from AMD sources, with limited flow from the pristine upstream portion of the basin, which is above treeline and which was completely frozen. As the seasons progressed, these pristine areas would warm and contribute higher pH water to Peru Creek, which in turn would increase the pH and thus cause a decrease in heavy metal concentrations. It is likely that this hydrologic dominance on Peru Creek played a significant role in controlling water quality conditions, which in turn affects the types of microorganisms capable of living there.

Proteobacteria Diversity

These communities, with the exception of Peru Creek during snow cover, were dominated by members of the *Proteobacteria* division. More than 40% of the sequences from this data set belong to the *Proteobacteria* division, with alpha and beta groups accounting for relatively similar proportions, followed by gamma and then delta. Within the *α-Proteobacteria,* the majority of the clones (100 of 185) do not fall into established groups, and little is known about their ecological function. *Acterobacteraceae* constitutes ~18% of the *α-Proteobacterial* sequences from this study. Within this family lies *Acidiphilium,* which is considered an important member of AMD environments. In this study, only one clone from this group was found in the Pennsylvania Mine site during snowmelt. This clone could have been introduced from

the mine tailings with snowmelt, but geochemical conditions were such that this species does not dominate in the effluent stream microbial community outside the mine. This may indicate that *Acidiphilium* was not an established population away from the mine site. Also, within this family are *Acetobacterium*, known as obligate anaerobes, which may suggest that anaerobic metabolic processes could occur.

Within the *β-Proteobacteria*, more than 22% of the clones from this study do not fall into a known group, and little can be inferred about their ecological function. Members of the *Burkholderiales* constitute more than 75% of the *β-Proteobacteria* found here, of which 51% fall into a specific group of clones from mainly Peru Creek and Snake River. The proportions in this group are highest during snowmelt, which may indicate enhanced growth conditions from constituents brought in during snowmelt or a flushing into the system of these bacteria from upstream portions of the watershed. Because there are no previously cultured representatives of this group, insight into their ecological impact is speculative at this point.

Although the proportion of *δ-Proteobacteria* is low, there are some metabolic capabilities in this subdivision that should be noted. *Desulfuomonadales* represents one-third of the sequences of *δ-Proteobacteria* from this study. This group is known to use sulfate and elemental sulfur as electron acceptors, and is well characterized. Members of this group can grow chemolithotrophically and autotrophically, as well as heterotrophically [Madigan 2000]. At the sites from this study, the majority of the sulfate reducers were found in Deer Creek during snowmelt and summer. Only one clone related to known members of this group was found in any of the acid-impacted

sites (Peru Creek during snowmelt). Because of the abundance of sulfate in these environments, it might be expected that sulfate reducers would be prevalent in AMD waters. This was not the case, which may indicate that a different group of sulfate-reducers are important in these systems or that other environmental conditions inhibited the growth of sulfate-reducing bacteria.

Gamma-proteobacteria are a very diverse group of organisms and include *E. coli*, *Pseudomonas* sp., *Enterobacteriaceae*, purple sulfur bacteria and *Vibro* sp. The majority of the sequences from this study fall into two groups: the Environmental Clone Group and the *Xanthomonadales*, with very little being known about the function of members in the former group. The majority of the sequences from acid-impacted sites fell into a separate group of clones within the Environmental Clone Group, which likely have a tolerance for the prevalent environmental conditions, such as low pH, heavy metals and high sulfate concentrations. Within the *Xanthomondales*, the sequences fall into two groups. Several sequences from Pennsylvania Mine and Snake River are closely related to clones from other AMD environments. The other group is composed primarily of clones found in Pennsylvania Mine. The function of these bacteria is not known because there are no cultured representatives related to our clones; however, members of the *Xanthomondales* group are considered to be similar to *Pseudomonas* [Madigan 2000], which may indicate similar metabolic capabilities, such as denitrification.

Acidobacteria

This group constitutes nearly 18% of the total sequences analyzed. With the exception of Peru Creek during snow cover, the relative proportions of *Acidobacteria* in these communities remains constant throughout the year. Nearly 25% of the clones sequenced from Peru Creek during snow cover were from *Acidobacteria-3* group, with 59% of the total snow-cover sample falling into this group. *Acidobacteria-3* contains other environmental clones that have been found in soil environments. Environmental clones from previous work often fall into the *Acidobacteria-1* grouping, which constitutes the majority of the remaining sequences from this study. The first isolate from the *Acidobacteria* division, *Acidobacterium capsulatum*, is a chemo-organotroph recovered from an AMD site [Kishimoto, et al. 1991], and it is not surprising that many of the sequences from these sites are related to another AMD microorganism. Some sequences from this study, mainly from Peru Creek and Snake River, fall into a separate group within *Acidobacteria*. Other member sequences had a close BLAST match with *Holophaga*, an anaerobic fermentor found in anoxic freshwater sediments, which may be another indication that anaerobic activity is occurring in these stream sediments. It is difficult to determine the ecological function of this group of bacteria, however, because there is very little known about its metabolic capabilities. This group is widely distributed in the environment and not restricted to acidic environments. Of the total sequences from Deer Creek, a near neutral pH stream, 7% fell into this group. It is interesting to note that the majority of the sequences from Deer Creek fall into the *Acidobacteria-4* and *Acidobacteria-6* groups. No sequences from the acid-

impacted sites fall into these groups, which may suggest a niche of bacteria that prefer higher pH conditions but that may be able to tolerate lower pHs because they are genetically related to other Acidobacteria.

Actinobacteria

The *Actinobacteria* division clones are dominated by members of the *Acidimicrobium*-related genus, with a few members from the *Corynebacterineae, Intrasporangiaaceae, Micrococcaceae* (*Arthrobacter* sp.) and *Propionibacterineae* families. Members from the *Acidimicrobidea* group include *Acidimicrobium ferrooxidans* and *Ferromicrobium acidiphilus,* which have both been shown to be acidophilic heterotrophs and iron oxidizers [Johnson 1998]. These organisms, and other clones closely related to them, have been shown to be important in AMD environments [Edwards, et al. 1999]. Therefore, it is not surprising to find them in high proportions in these acid-impacted streams. It is, however, unclear why members of the *Acidimicrobium* genus represent 5% of the overall clones from the pristine Deer Creek, where the pH range is between 6.21 and 6.36. These organisms seem to be most prevalent in the microbial community samples from this study during snow cover.

Other Bacterial Divisions

Several of the clones are related to clones found in divisions established with members from Yellowstone National Park's Obsidian Pool [Pace 1996]. Members of these divisions (OP3, OP5, OP8, OP10, OP11) are found in a variety of environments.

It has been suggested that because bacteria from these divisions are widespread in the environment, they may play a significant ecological role [Hugenholtz 1998]. In this study, bacteria from these divisions were only found during snowmelt and summer, possibly suggesting that they are brought in with snowmelt runoff, persist during summer and die off during snow cover because of the colder winter temperatures or some other environmental factor. Until sequences for cultured representatives can be established and metabolic processes characterized, it is difficult to predict what the bacteria's role in the environment might be.

Verrucomicrobia clones were found at all sites, which might be expected because members of this division are widespread in the environment [Hugenholtz 1998]. One clone from the *Deinoccoccus* division, found in the Deer Creek snow-cover sample, was most closely related to a clone from South Pole snow (I had to get Antarctica into this dissertation somehow), which could indicate some affinity for pristine, cold environments. Clones from the division *Chlamyadie* were found in the Pennsylvania Mine and Deer Creek and were most closely related to clones classified as endosymbionts of *Acanthamoeba* sp., suggesting the presence of *Acanthamoeba* sp., a common soil protozoan.

Clones grouped into *Bacteriodes* made up 3.9% of the total sequences examined, of which *Saprospiraceae* is the dominant family. Members of *Saprospiraceae* are anaerobic and are associated with animal feces [Madigan 2000]. Their presence at these sites again indicates either that anaerobic conditions for growth existed in zones

in sediments or that they were introduced into the streams from terrestrial or aquatic fecal material.

Chlorobi, also known as green-sulfur bacteria, are photosynthetic strict anaerobes that can use sulfur as an electron donor. Their presence suggests that anaerobic conditions exist and that anaerobic metabolic processes can occur in these environments. *Chlorolexi*, green nonsulfur bacteria, were originally thought to be only thermophilic, but with recent phylogenetic studies, they have been detected in soil, sediment and aquifer environments [Madigan 2000]. *Chloroflexus*, perhaps the most studied member of the *Chloroflexi* division, forms thick microbial mats and can support photoautotrophy where H_2S or H_2, along with CO_2, provide energy for metabolism [Madigan 2000]. The majority of the clones found at these sites fall into a separate group within *Chloroflexi*, of which there are no cultured representatives. These results suggest the existence of acid-tolerent photoautotrophs. The highest clone abundances from this group occurred during snowmelt, suggesting that they are brought in with snowmelt runoff, possibly from alpine regions.

Planctomycete-related clones were found at all sites, with the most clones in Deer Creek during snow cover. These bacteria are considered facultative aerobic chemo-organotrophs that can grow by fermentation or respiration of sugars. This group is phylogenetically unique and encompasses organisms capable of anaerobic ammonia oxidation [Thamdrup and Dalsgaard 2002]. By mineralization of marine snow particles, they have a profound impact on global biogeochemistry and climate, affecting exchange processes between the geosphere and the atmosphere [Fuerst 1995].

Eucaryotes and Cyanobacteria

Eucaryotes were only present in the Pennsylvania Mine and Snake River during snow cover. Both of these sediments were dominated by members of the *Chryosophyte* class, which can make up a major component of the planktonic community, particularly during cold months [Sze 1986], which may explain the high percentage seen during snow cover in these streams. In addition to photosynthesis, these organisms can obtain energy from dissolved organic matter [Sze 1986], which may be an important function during much of the year when these streams are covered by several meters of snow. The Eucaryotes from the Pennsylvania Mine site are related to the *Synura* genus, members of the *Chrysophyte* class, which are colonies of cells found in freshwater environments and which have delicate siliceous scales and no mucilage sheath. Because of their colonial nature, it is difficult to distinguish whether these Eucaryotes truly dominate this community or whether a single colony was present in the limited, random sediment samples collected. However, the fact that sediment samples were collected in triplicate and the DNA was pooled from the samples reduces the possible effect any one colony may have had on the total microbial population sampled.

It is important to note the sequences that fell into the *Cyanobacterial* division: they were all chloroplasts, with the majority from Euglena. In general, the percentage of chloroplasts increased from snow cover to summer in all stream sediments examined, corresponding to the amount of incident sunlight reaching the stream channel, which increases from snow cover to summer. Euglena algae tend to be

abundant in waters with high organic content. Both the Snake River and the Pennsylvania Mine streams have low DOC concentrations; however, sediment organic content was not measured and may have been significant. It has been documented that iron can complex with organic matter [Marchand and Silverstein 2002, McKnight, et al. 2002] and thus be available in the iron oxide sediments that have precipitated out of the stream to lie on top of the streambed.

Archaea

Based on observations of a few cultivated members of this domain, it was thought that Archeae were restricted to extreme environments. With advances in molecular microbiology, however, this perception is changing. Members of the *Ferroplasma* group made up 85% of the total population in a biofilm sample from the Richmond Mine, part of the Iron Mountain Mine [Edwards, et al. 2000]. In this study, the lack of abundant archaeal clones from the two AMD-impacted streams suggested that Archaea may not be as important in downstream microbial communities. Clones found in the Snake River during snowmelt are closely related to other *Crenarchaeota* clones found in soils, forest soils and alpine tundra. The sampling site for Snake River was below treeline and, therefore, not considered a tundra environment. It is likely that these clones were brought in with snowmelt runoff from the alpine tundra region of the watershed. Thus, they possibly would not persist throughout the summer and snow-cover seasons. The clones found during snow cover and summer were closely related to deep subsurface clones. It may be possible that these clones were transported

into the stream system by hyporheic flow because groundwater in these environments is very shallow. Although Archaea were only 0.6% of the Deer Creek sample, it is interesting to mention that these two *Euryarchaeaota* were most closely related to clones found in a contaminated aquifer and an anaerobic digester, environments that are not usually considered "pristine." It was interesting to find that archaea from a pristine stream are close relatives of clones found in anthropogenically impacted environments. Because there are no cultured representatives of these groups, it is difficult to determine their ecological function in these systems.

Implication for Denitrification

It has been estimated that denitrifiers typically make up 0.1–5% of the total culturable population in mineral soils [Tiedje 1994]. It has also been stated that approximately 99% of microorganisms cannot be cultured [Pace 1996]. Assuming that this percentage is consistent among the 99% of microorganisms that are not culturable, there would be a large number of species capable of denitrification.

Complete reduction of nitrate to nitrogen gas was observed with sediments from the acid-impacted streams as well as the pristine stream sites (see Chapter 5). In a first attempt to understand which microorganisms might be responsible for this metabolism, 16S rRNA techniques were used as a tool to identify previously known denitrifiers. None of the known major groups of denitrifiers were identified using this technique. Earlier studies have demonstrated that denitrification can occur in these acid-impacted mountain streams. Thus, there are species present in these sediments that have not yet been identified as denitrifiers.

Results from more than 1,345 clone sequences did not show any members of the *Pseudomonas* group, which illustrates that the most abundant soil denitrifiers (using culture-based methods) are not responsible for the process of denitrification in these sediments. Of the other common denitrifiers, only members related to *Bacillus* and *Flavobacterium* were detected in our clones, and only in low abundance. Thus, although they may be involved in the denitrification process, these common denitrifiers do not seem to be contributors to denitrification at these sites. *Bradyrhizobium* sp. was detected only in Peru Creek at all seasons, in Deer Creek during summer and in Snake River during snow cover. These organisms could denitrify in those sites. However, because no members of the *Bradyrhizobium* genus were found at the other sampling times or at Pennsylvania Mine, the presence of other organisms that are also denitrifying is likely. Two clones from the *Rhizobiales* group, which may be denitrifiers, were found in Deer Creek during snow cover. Members of the *Flexibacteraceae* family found in Pennsylvania Mine and Peru Creek also have the capacity for denitrification. One clone from Deer Creek under snow cover was found to be related to *Hyphomicrobiium*, a known nitrate reducer. The clones in the *Xanthomonadales* group are related to a previously identified Fe^{2+} denitrifying clone [Straub and Buchholz-Cleven 1998], which may suggest that this group is capable of denitrification using Fe^{2+} as an electron donor.

Statistical correlations between the number of clones from each division and denitrification potential rate constants were conducted. The results suggest that denitrifiers might be members of the *α-Proteobacterial*, *Actinobacteria* and *Chloroflexi* divisions. In particular, these correlations were higher among the acid-impacted sites.

Because there were no significant correlations among specific groups within these divisions and rates, it is likely that acid-tolerant denitrification may be widespread within these bacterial divisions and not an uncommon metabolism.

Advances in microbial identification techniques, mainly the use of 16S rRNA genes, have enabled identification of entire communities of microorganisms. However, these advances did not provide clear evidence of which microorganisms denitrified. Further analysis of these sites using functional genes to examine denitrification enzyme diversity may provide more insight into denitrifying populations in these environments.

Implications for Other Biogeochemical Nitrogen-Cycling Processes

Members of the *Planctomycetes* are known to anaerobically oxidize ammonium coupled with nitrate reduction to nitrogen gas [Thamdrup and Dalsgaard 2002]. The presence of clones from this group may suggest that anaerobic ammonia oxidation (AMMONOX) is another nitrogen metabolism closely related to denitrification that could occur in these environments. Many sulfate reducers can also grow on nitrate as an electron acceptor, producing ammonium rather than nitrogen gas [Moura 1997, Yamamoto-Ikemoto 2000]. Either of these may be alternate nitrogen-cycling processes to explore in these environments.

Clones related to *Nitrospira* were found, but only in Deer Creek. These organisms are known as nitrifiers, and because nitrate is present in these stream waters, it is possible that nitrification is occurring. Organisms responsible for nitrification in these streams warrant further investigation.

Nitrogen fixation is another nitrogen-cycling process that has not been explored in AMD environments. Clones related to *Clostridium* sp., of which members are known to fix nitrogen, are present in these sites and may be capable of nitrogen fixation under acidic conditions. Another player in nitrogen fixation may be nitrogen-fixing algae. Clones from this study show large populations of algae during snow cover in two of the acidic sites. The probability of acidophilic or acid-tolerant nitrogen fixation is high in these environments. To date, it is yet unclear if this process is occurring and which microorganisms may be responsible.

Conclusions

Twenty-one different divisions of bacteria were represented in the 1,345 clones sequenced from the acid-impacted mountain stream sediments and the pristine sediment. Eucaryotes and Archaea were also present. Overall, the communities were dominated by *Proteobacteria*, with the exception of Pennsylvania Mine, Peru Creek and Snake River during the snow cover season. *Acidobacteria* were present in high percentages of the total clones from Peru Creek and Snake River. Deer Creek had more clones belonging to the *Acidobacteria* division than Pennsylvania Mine, the most acidic site examined.

The major bacteria genera usually identified with denitrification were not found in the acidic and pristine mountain stream sediment population, even though biological denitrification occurred in all these sediments. This suggests that a different group of microorganisms, possibly associated with the *α-Proteobacteria*, *Actinobacteria*

and/or *Chloriflexi* groups, is capable of this process. Further research in these environments, and a more detailed examination of denitrifiers, perhaps with functional genes, is suggested.

CHAPTER 7: CONCLUSIONS

Conclusions

This research is relevant to both basic and applied stream ecology and to nutrient cycling. A significant finding of this work was that microorganisms capable of denitrification (the reduction of nitrate to nitrogen gas) are present and active in acid-impacted mountain streams throughout the year. Therefore, the conclusions below are made with respect to the environmental aspects of the associated experimental results.

- Denitrification was carried out by microorganisms from acid-impacted stream sediments with an ambient pH range of 2.6 to 5.6. Nitrate was reduced completely to N_2 in laboratory microcosms regardless of the season in which sediment were collected. The highest denitrification potential rates were obtained from sediment collected under snow cover. First-order specific denitrification potential rate constants ranged from 0.03 to 1.54 μmoles-NO_3-N/g-sediment/day, which is significantly higher than rates reported by other researchers in circumneutral soils. Denitrification in anoxic microcosms began immediately. No nitrite was detected during denitrification, and 89–97% of added nitrate was recovered as nitrogen gas or residual nitrate.
- Overall, the highest denitrification potential rates were measured in sediments from a pristine and a naturally acidic stream (1.51 and 1.54 μmoles-NO_3-N/g-sediment/day, respectively), as compared with the maximum rates observed in a

mine effluent and an AMD-influenced stream (0.22 and 0.43 g-NO_3-N/g-sediment/day, respectively).

- During denitrification, the pH of microcosm sediment slurries increased from 0.4 to 1.5 units. As the pH increased, the concentration of several dissolved heavy metals (Al, Cu and Zn) decreased. Denitrification potential rates by sediments from acid-impacted streams were correlated neither to ambient pH in the range of 2.6 to 5.6 nor to the concentration of dissolved heavy metals, implying that denitrifying populations were tolerant to acidity and metal ions.
- Raising the pH of microcosm sediment slurries from the ambient stream value of 4.1 to as high as 6.0 did not increase the denitrification potential rate. However, decreasing the pH to 2.6 did result in a lower denitrification potential rate until slurries reached pH ~3.75, suggesting that the denitrifying population was acclimated to in situ stream pH conditions.
- Microcosm denitrification potential rate constants were most strongly correlated to the DOC concentration in stream water for sediment samples from all locations in all seasons (R^2 = 0.867, p = 0.01), indicating that denitrification in these environments was primarily carried out by heterotrophic organisms. Addition of glucose or soluble natural organic matter to microcosms stimulated denitrification, suggesting that organic carbon was a rate-limiting substrate.
- Nitrous oxide (N_2O) was detected in all streams at all seasonal sampling times in the concentration range of 2.9 to 16.3 nM (0.04 to 0.23 µg-N/L) and was somewhat correlated with the microcosm denitrification potential rate, although

the relation was not significant at the $p < 0.05$ level ($R^2 = 0.586$, $p = 0.097$). Interestingly, the field measurements of nitrate were not at all correlated with the microcosm denitrification potential rate ($R^2 = 0.039$, $p = 0.92$). Nitrous oxide is a common product of incomplete denitrification, and its relation to microcosm denitrification potential rates is evidence that the laboratory microcosm sediment slurry method was probably indicative of denitrification activity in the streams.

- The denitrification potential rates in microcosms decreased significantly (50–80%) with the addition of ferrous and ferric iron in concentration range of 50 to 200 mg-Fe/L, with ferric iron addition producing the greater inhibition effect. Complexation of limited organic matter by ferric and, to a lesser degree, ferrous iron may explain the rate reduction and was consistent with carbon limitation of denitrification. However, this was not proven experimentally.

- Analysis of 12 samples of sediment genetic material from 4 streams resulted in 1,345 clone sequences (847 unique clones), representing microorganisms from the *Bacterial*, *Eucaryotic* and *Archaeal* domains. Bacterial clones included representatives of 21 different divisions, 40% of the 52 known bacterial divisions. Genetic analysis indicated that *Proteobacteria* dominated sediment microbial communities from AMD, naturally acidic and circumneutral streams, with three exceptions: Eucaryotes dominated in the Pennsylvania Mine effluent and in the naturally acidic stream (Snake River) in sediment samples taken during snow cover, and members of the *Acidobacterial* division made up 75% of the clones sequenced from the AMD-impacted stream (Peru Creek) during snow cover.

- None of the 1,345 clones sequences was from major groups of bacteria shown to be capable of denitrification in agricultural and natural soils and sewage (e.g., *Pseudomonas*). Microcosm denitrification potential rates did correlate positively with several groups that were represented by sediment clones: *α-Proteobacteria*, *Actinobacteria* and/or *Chloriflexi*, suggesting that denitrification is a widely distributed metabolism shared by bacteria present in the harsh environments of AMD- and ARD-impacted streams.

Broader Impacts

Nitrogen budgets have been conducted for the Rocky Mountains, paying particular attention to the fate of increased nitrogen deposition. These models have not taken into account biogeochemical nitrogen cycling in acid-impacted streams. The results from this research can be used to construct more complete estimates of the fate of nitrate in mountain streams, particularly because denitrification potential rate constants from the streams surveyed are higher than previously thought. Because AMD-impacted streams are found around the world, denitrification activity in these streams may need to be considered for nitrogen budgets in other alpine areas of the world. Furthermore, because the process is heterotrophic in nature, denitrification in acid-impacted mountain streams may be important when determining carbon budgets.

Using the most common molecular tool available for assessing microbial communities, no common denitrifiers were sequenced. Using 16S rRNA techniques is a good method to characterize the overall community, but additional experimental

methods also need to be conducted when assessing denitrification activity, especially in environments where the process has not been examined prior.

Denitrification may have the potential to be used as a remediation strategy for AMD-impacted streams, because it increases the pH of surrounding waters, thus causing many heavy metals to precipitate.

Results from this research provide evidence that biogeochemical nitrogen processes in acidic environments are occurring. This adds to our understanding of the global nitrogen cycle, as well as of the metabolic capabilities of microorganisms living in this extreme environment. This understanding adds to our limited knowledge of biogeochemical nitrogen cycling in extreme environments as well as effects of multiple stressors on the microbial community. Process studies such as this are needed to help characterize metabolic capabilities of microorganisms living in extreme environments. The combination of data from 16S rRNA sequences and process studies could greatly enhance our understanding of extreme environments and give insights into more "typical" ecosystems.

Further Research Suggestions

The results from this research have opened up a new aspect of nitrogen cycling and added to our understanding of acid mine drainage. Some suggestions for additional studies are listed below.

- Addition of iron had a negative effect on denitrification in acid-impacted streams studied here. The reasons for this are unclear. Experiments to further examine

iron speciation coupled with denitrification intermediates should be conducted. Furthermore, it is known that iron complexes with dissolved organic carbon on stream precipitates. It is likely that there is a compounding effect of iron, carbon and nitrogen species in these systems that should be explored, both by laboratory incubations and in situ assessments.

- In situ denitrification studies should be conducted. These should include tracer tests with nitrate addition to examine in-stream uptake of nitrate, along with the influences of hyporheic exchange.
- The hydrograph in these mountain streams is dominated by a large pulse of water, dissolved constituents and particulates entering during snowmelt, accompanied by an increase in nitrate. The response of the microbial community during this time is important to examine. Many mountain catchments are sources of drinking water, and excess nitrate could cause human health or ecological problems, such as eutrophication. To predict treatment processes and facilities needed to treat additional nitrate entering through atmospheric deposition, we should first understand the processes controlling water quality, especially during times when facilities may become overwhelmed, such as during snowmelt. This could be done using laboratory studies, in situ monitoring or more detailed enzyme analyses.
- AMD-impacted streams may be an important environment to examine aspects of autotrophic denitrification, especially when and where carbon is limited. With the number of possible inorganic electron donors in these systems, the likelihood that

bacteria (or archaea) capable of using these alternative donors is high. Experiments should be conducted to examine these processes.

- Given the absence of classic denitrifying bacteria in these streams, more work should be conducted to identify the microorganisms responsible for denitrification. This could be done using enrichment cultures, combined with 16S rRNA techniques. Another means of examining denitrification at a molecular level in these systems would be the use of functional gene analysis.
- The absence of nitrite, an intermediate of denitrification, is an area that warrants further investigation. Nitrite may be chemically reactive at these low pHs, which could produce HNO_2. The pathway of nitrate to nitrogen gas needs to be explored, especially with respect to the cellular mechanisms involved with nitrate reduction.
- This study on denitrification in AMD-impacted streams highlights the limited knowledge regarding nitrogen cycling in these environments as a whole. The possibilities for additional research for other nitrogen metabolisms are numerous and warrant further exploration.

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Appendix I: Preliminary Data and Discussion

Preliminary results suggest that denitrifying bacteria are present in several acid mine drainage streams. Three sites were examined to determine if denitrifiers were present in AMD sediments. The preliminary sampling site was Gamble Gulch, located south of Rollinsville, CO in the Rocky Mountain Front Range. Gamble Gulch drains the abandoned Tip Top Mine and has the characteristic low pH (ranges from 3.75 to 4.5) and high concentrations of heavy metals. A nearby circum-neutral pH stream, Jenny Lind Gulch, was chosen as a comparison site. A mine in Summit County, CO was also examined because of the variety of drainage streams flowing through the mine site, with pH values ranging from 3.25 to 5.0, from tunnels, waste rock and tailings.

Sediment and water were collected from each site and immediately analyzed for denitrification potential using the acetylene block technique [Tiedje 1994]. Assays were conducted in 125-mL flasks containing approximately 30 grams of dry weight sediment and 50 mL stream water. No nitrate was detected in the natural waters, so it was added to obtain an initial flask concentration of 0.7 mM (10 mg/L NO_3-N). Oxygen-free purified acetylene was added to each flask to block N_2 formation and nitrous oxide (N_2O) concentrations were measured by gas chromatography. Selected natural conditions (pH, substrate availability) were varied to determine how denitrification could be suppressed or enhanced in AMD waters.

Comparison of assays with untreated and sterilized Gamble Gulch sediment showed that after 75 hours of incubation no N_2O was produced in the autoclaved sediment while 27 nmoles of N_2O /g sediment was steadily produced from the ambient

sediment. This indicated that denitrifiers were present in the AMD sediment from Gamble Gulch and capable of denitrification. Only a fraction of the nitrate was converted to N_2O. Further analysis of the fate of nitrate in these microcosms needs to be explored.

To determine how the denitrification rate compared to that of a nearby pristine stream that was not impacted by AMD, sediment and surface water from Jenny Lind Gulch were analyzed at ambient pH of 7.0 and at an adjusted pH of 4. Gamble Gulch sediment and surface water were also run at ambient pH of 4 and an adjusted pH of 7. Results from this experiment are shown in Figure A1-1. After a period of lag, the denitrification potential in the pristine sediment adjusted to an acidic pH and was not significantly different from the pristine sediment at its ambient pH. This suggests that the denitrifying bacteria in pristine sediments may not be adversely affected long-term by acidic conditions. The AMD sediment at ambient pH and adjusted to pH 7 do not have considerably different denitrification potentials. The average denitrification rate in pristine sediment was 37.9 nmoles N_2O/g-sediment/day which is significantly higher than the 3.4 to 19.2 nmoles N_2O/g-sediment/day denitrification rate in the AMD sediment. This could be a result of more electron donor being available in the pristine sediment, more denitrifying bacteria present in the pristine sediment, pH, metal toxicity, or a difference in nitrogen intermediates that are generated. This difference in the denitrification in these two diverse streams needs to be explored further.

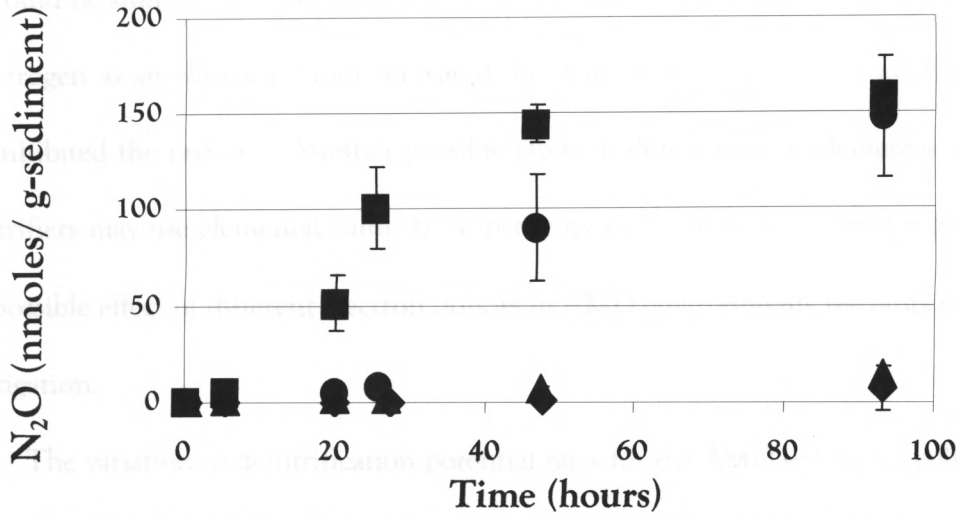

Figure A1-1. Denitrification Potential in Pristine vs. AMD Sediment

■ pristine sediment at ambient pH, ● pristine sediment adjusted to pH 4, ▲ AMD sediment at ambient pH and ◆ AMD sediment adjusted to pH 7.

Carbon is often an electron donor in the denitrification process [Devlin 2000, Kelso 1999]. Because carbon is usually limited in AMD streams, experiments were conducted to determine which carbon sources or other possible electron donors stimulated denitrification in AMD streams. Denitrification assays were performed using the same procedure outlined above with several carbon sources added at 5mM concentrations. Results indicated that acetate and nutrient broth had the greatest impact. No denitrification was observed with the addition of propionate and the addition of glucose hindered the production of N_2O. Denitrification rate potentials for the various electron donor additions are shown in Table A1-1.

A second set of tests were conducted with AMD sediment samples collected near the same location as sediment obtained for the organic electron donor experiment. These sediments were used to assess the influence of inorganic electron donors. Results from this experiment showed the presence of denitrifying bacteria,

that could be stimulated by the addition of non-organic electron donors. The addition of hydrogen as an electron donor increased the denitrification potential, while added Fe^{2+} inhibited the process. Another possible electron donor may be elemental sulfur. Denitrifiers may use elemental sulfur from pyrite as an electron donor in this process. The possible effect of different electron donors in AMD environments warrants further investigation.

The variation in denitrification potential rates for the AMD sediments collected on the same day from nearby locations indicates a spatial patchiness of denitrifiers in the stream sediments. Further analysis of the distribution of denitrifiers may lead to a better understanding of conditions these bacteria prefer, possibly a microenvironment that is more conducive to their existence and growth.

Table A1-1. Denitrification potential rates by various electron donors.

Organic Electron Donor	Denitrification Potential Rate (nmole N_2O/day)	Inorganic Electron Donor	Denitrification Potential Rate (nmole N_2O/day)
No donor added	4.15		
Acetate	6.96	No donor added	33.53
Nutrient Broth	5.39	Hydrogen	58.22
Glucose	1.67	Fe^{2+}	14.30
Propionate	0.00		

To investigate the possibility of denitrification as a remediation strategy for AMD streams, sediments were obtained from an active molybdenum mine in Summit County, CO. At this site, four samples were collected from various drainages at the mine. Denitrification potential assays were conducted and initial and final pH values recorded. Results from this experiment are shown in Figure A1-2. McNulty 1 and 2 are from McNulty Gulch that drains from a tailings pile. The samples were taken about 3 feet apart. Pipe drain is from an area where a drainage pipe collecting drainage

from several tailings pile, empties into a ditch. The pond is nearby and un-impacted by AMD. The pH in McNulty 1 increased from 3.25 to 5.33 during a 100-hour incubation. The final pH is well above the soluble range for many metals and many of the metals in solution may have precipitated during the incubation, as indicated by the formation of orange and white deposits on the sediments. The pH in McNulty 2 and the Pipe Drain also increased. It is likely that after a longer incubation, the denitrification process would have caused the pH to rise to a similar level. The difference in rates in the two McNulty samples again point to possible spatial patchiness of denitrifiers in these stream systems. The increase in pH in the pond sample was expected because denitrification generated alkalinity. These results indicate that with further study enhanced denitrification in AMD streams may be a viable remediation strategy.

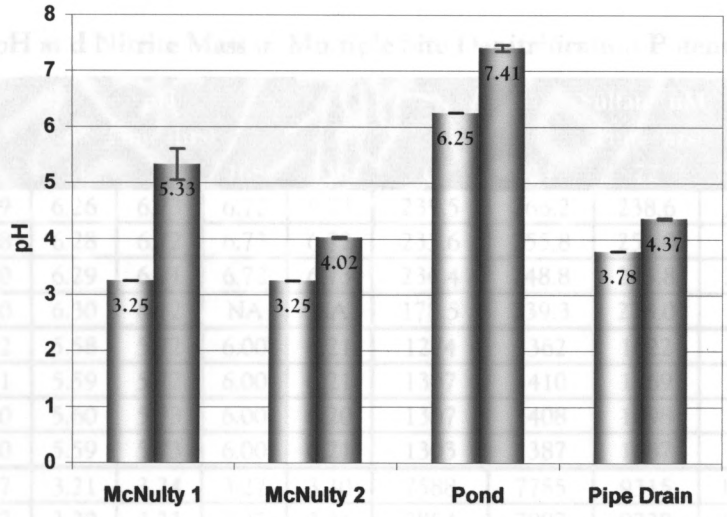

Figure A1-2. Effects of denitrification on pH. Lighter bars on left are initial pH and darker bars on right are final pH values after 100 hours. Error bars depict standard deviation of triplicate samples.

Appendix II: Raw data from Chapter 4

Table AII-1. Nitrate Concentrations (μM) for Multiple Site Microcosms

Flask	Sediment Dry Weight (mg)	Time (hours)				
		0	24.2	56.7	76.7	148.6
Pennsylvania Mine 1	35.47	356.4	251.5	230.1	209.3	154.3
Pennsylvania Mine 2	33.88	356.4	250.6	237.5	213.9	130.8
Pennsylvania Mine 3	34.42	356.4	278.4	240.5	207.8	134.0
Pennsylvania Mine 4	35.12	356.4	251.5	229.9	202.6	120.8
Cinnamon Gulch 1	38.55	336.2	256.0	171.8	31.83	32.64
Cinnamon Gulch 2	42.51	336.2	216.4	165.2	5.00	5.00
Cinnamon Gulch 3	44.12	336.2	251.2	155.3	5.00	5.00
Cinnamon Gulch 4	42.61	336.2	239.8	168.2	5.00	25.88
Snake River 1	46.52	332.5	282.4	251.3	211.2	96.44
Snake River 2	52.12	332.5	292.3	230.5	206.2	79.38
Snake River 3	47.06	332.5	275.2	242.2	197.9	97.03
Snake River 4	49.02	332.5	265.6	216.3	217.2	80.3
St. Kevin Gulch 1	47.78	351.2	325.6	311.4	305.4	299.9
St. Kevin Gulch 2	49.66	351.2	312.1	313.9	304.4	258.3
St. Kevin Gulch 3	54.00	351.2	310.3	304.3	289.6	251.7
St. Kevin Gulch 4	58.14	351.2	286.0	279.9	255.9	174.2
Tailings Pile Spring 1	59.56	376.2	286.3	278.8	254.0	252.9
Tailings Pile Spring 2	49.25	376.2	304.5	312.0	276.1	287.1
Tailings Pile Spring 3	54.15	376.2	291.1	275.8	258.3	177.2
Tailings Pile Spring 4	50.79	376.2	281.1	276.3	257.9	263.6

Table AII-2. pH and Nitrite Mass in Multiple Site Denitrification Potential Microcosms

	pH					Sulfate (μM)				
	Time (hrs)					Time (hrs)				
Flask	0	24	72	168	336	0	24	72	168	336
Deer 1	5.99	6.26	6.73	6.72	6.73	239.5	266.2	238.6	258.3	463.5
Deer 2	6.08	6.28	6.72	6.73	6.72	233.6	255.8	251.0	348.9	391.1
Deer 3	6.10	6.29	6.71	6.72	6.73	234.4	248.8	245.8	284.1	361.3
Deer 4	6.10	6.30	6.72	NA	NA	178.5	239.3	278.0	NA	NA
Snake 1	5.32	5.58	5.72	6.00	6.21	1274	1362	1622	1931	3136
Snake 2	5.31	5.59	5.72	6.00	6.21	1307	1410	1769	2277	3522
Snake 3	5.30	5.60	5.73	6.00	6.20	1307	1408	1698	1886	3904
Snake 4	5.30	5.59	5.73	6.00	6.21	1303	1387	1687	2048	3246
Penn 1	3.17	3.21	3.24	3.27	3.30	7588	7755	9315	11800	17020
Penn 2	3.17	3.20	3.23	3.27	3.31	7854	7997	9228	11260	14880
Penn 3	3.17	3.21	3.24	3.28	3.31	7990	8159	9654	12030	16280
Penn 4	3.18	3.20	3.24	3.27	3.31	7778	8137	9814	11960	15710
Peru 1	4.62	4.67	4.73	4.88	4.92	1024	1147	1520	2087	3871
Peru 2	4.62	4.68	4.73	4.88	4.92	1072	1180	1541	1869	2611
Peru 3	4.62	4.68	4.73	4.88	4.92	1054	1147	1380	1705	2372
Peru 4	4.62	4.68	4.73	4.88	4.92	1079	1196	1424	1737	2301

Table AII-3. Total N_2 Mass in Multiple Sites Denitrification Potential Microcosms

	Time (hours)				
Flask	0	24.2	56.7	76.7	148.6
Pennsylvania Mine 1	0.00	2.94	6.19	14.21	23.78
Pennsylvania Mine 2	0.00	9.85	13.77	16.48	23.86
Pennsylvania Mine 3	0.00	4.84	9.80	14.92	26.70
Pennsylvania Mine 4	0.00	13.94	15.37	16.86	28.85
Cinnamon Gulch 1	0.00	10.80	14.13	24.16	30.99
Cinnamon Gulch 2	0.00	6.87	15.05	22.05	31.49
Cinnamon Gulch 3	0.00	8.20	15.04	28.42	30.64
Cinnamon Gulch 4	0.00	5.99	15.26	26.55	31.62
Snake River 1	0.00	3.46	9.77	14.65	26.06
Snake River 2	0.00	2.56	9.08	14.85	28.48
Snake River 3	0.00	1.00	10.43	13.07	24.13
Snake River 4	0.00	1.98	12.84	13.73	27.95
St. Kevin Gulch 1	0.00	3.99	8.13	11.81	16.63
St. Kevin Gulch 2	0.00	1.80	6.77	12.58	20.03
St. Kevin Gulch 3	0.00	0.58	9.07	12.16	20.41
St. Kevin Gulch 4	0.00	1.80	7.30	9.50	23.85
Tailings Pile Spring 1	0.00	8.36	7.79	13.03	19.51
Tailings Pile Spring 2	0.00	6.76	8.97	12.42	19.98
Tailings Pile Spring 3	0.00	3.47	5.95	12.47	24.27
Tailings Pile Spring 4	0.00	6.63	8.23	14.79	18.12

Table AII-4. pH and Nitrite Mass in Multiple Site Denitrification Potential Microcosms

	pH					NO_2^- (nmoles)				
	Time (hrs)					Time (hrs)				
Flask	0	24.2	56.7	76.7	148.6	0	24.2	56.7	76.7	148.6
Penn 1	3.23	3.31	3.48	3.50	4.05	0.93	11.3	5.74	4.81	3.23
Penn 2	3.23	3.30	3.46	3.51	4.08	0.93	15.2	9.02	6.93	2.98
Penn 3	3.23	3.32	3.46	3.48	4.06	0.93	18.9	17.7	9.40	16.1
Penn 4	3.23	3.30	3.50	3.52	4.07	0.93	12.6	11.0	14.9	12.5
Cinn 1	4.12	4.19	4.24	4.29	4.32	0.93	12.7	68.7	17.4	0.89
Cinn 2	4.12	4.17	4.27	4.30	4.35	0.93	21.7	6.45	65.5	1.14
Cinn 3	4.12	4.18	4.25	4.32	4.37	0.93	31.2	237	34.9	0.74
Cinn 4	4.12	4.20	4.20	4.32	4.37	0.93	22.1	57.4	28.0	0.61
Snake 1	4.54	5.01	5.48	5.74	6.01	0.93	563	717	1120	8.75
Snake 2	4.54	5.03	5.43	5.83	6.04	0.93	473	1050	1050	4.01
Snake 3	4.54	5.02	5.42	5.76	6.02	0.93	550	1010	972	3.18
Snake 4	4.54	5.04	5.45	5.80	6.03	0.93	788	1130	1260	5.07
St. Kevin 1	4.20	4.28	4.38	4.52	4.75	0.93	1.64	6.64	11.7	19.5
St. Kevin 2	4.20	4.29	4.45	4.53	4.73	0.93	1.51	16.0	36.5	11.0
St. Kevin 3	4.20	4.27	4.40	4.48	4.72	0.93	1.38	13.2	14.2	18.8
St. Kevin 4	4.20	4.28	4.54	4.61	4.74	0.93	NA	63.8	71.9	10.2
Tailings 1	2.60	2.65	2.71	2.74	2.95	0.93	2.36	2.55	2.57	1.52
Tailings 2	2.60	2.64	2.72	2.74	2.96	0.93	2.25	3.43	1.65	1.78
Tailings 3	2.60	2.66	2.71	2.72	2.97	0.93	2.27	1.76	1.49	1.35
Tailings 4	2.60	2.65	2.72	2.73	2.99	0.93	2.30	3.10	2.13	0.85

Table AII-5. Dissolved total Al, Cd and Co concentrations during denitrification for multiple AMD-impacted sites

	Aluminum (mg/L)					Cadmium (mg/L)					Cobalt (mg/L)				
	Time (hours)					Time (hours)					Time (hours)				
	0	24	52	77	149	0	24	52	77	149	0	24	52	77	149
Penn 1	18.000	12.000	11.000	8.700	8.900	0.110	0.110	0.120	0.120	0.130	0.063	0.076	0.079	0.081	0.086
Penn 2	18.000	12.000	9.600	8.800	8.700	0.110	0.120	0.120	0.120	0.130	0.063	0.076	0.079	0.079	0.087
Penn 3	18.000	12.000	9.600	9.000	9.100	0.110	0.120	0.120	0.120	0.130	0.063	0.076	0.079	0.082	0.089
Penn 4	18.000	12.000	9.600	8.400	9.700	0.110	0.110	0.120	0.110	0.130	0.063	0.073	0.079	0.077	0.089
Cinn 1	3.400	2.100	1.100	0.930	1.100	0.006	0.013	0.009	0.006	0.010	0.006	0.034	0.042	0.047	0.083
Cinn 2	3.400	1.500	1.500	1.000	0.950	0.006	0.008	0.027	0.006	0.008	0.006	0.032	0.037	0.043	0.074
Cinn 3	3.400	2.000	0.980	0.900	0.980	0.006	0.008	0.007	0.005	0.007	0.006	0.039	0.050	0.050	0.079
Cinn 4	3.400	1.400	1.100	0.970	0.870	0.006	0.007	0.006	0.006	0.008	0.006	0.034	0.037	0.039	0.066
Snake 1	4.400	0.500	0.250	0.300	0.070	0.005	0.001	0.001	0.001	0.001	0.014	0.008	0.003	0.003	0.003
Snake 2	4.400	0.610	0.150	0.070	0.070	0.005	0.001	0.001	0.001	0.001	0.014	0.003	0.003	0.003	0.003
Snake 3	4.400	0.310	0.070	0.180	0.070	0.005	0.001	0.001	0.001	0.001	0.014	0.003	0.003	0.003	0.003
Snake 4	4.400	0.800	0.070	0.070	0.230	0.005	0.001	0.001	0.001	0.001	0.014	0.003	0.003	0.003	0.003
St. Kevins 1	2.100	2.000	1.400	1.500	0.830	0.016	0.029	0.033	0.044	0.068	0.009	0.015	0.016	0.022	0.035
St. Kevins 2	2.100	1.800	1.400	1.200	0.740	0.016	0.024	0.033	0.040	0.057	0.009	0.012	0.017	0.021	0.032
St. Kevins 3	2.100	2.100	1.500	1.400	0.790	0.016	0.024	0.034	0.041	0.053	0.009	0.012	0.018	0.026	0.032
St. Kevins 4	2.100	1.600	1.200	0.880	0.610	0.016	0.026	0.037	0.040	0.055	0.009	0.016	0.024	0.029	0.038
Tailings 1	8.700	7.000	7.100	6.500	7.100	0.330	0.290	0.270	0.240	0.220	0.061	0.065	0.072	0.066	0.069
Tailings 2	8.700	7.000	6.700	7.700	8.300	0.330	0.290	0.250	0.250	0.240	0.061	0.062	0.062	0.066	0.070
Tailings 3	8.700	6.900	6.700	7.300	8.300	0.330	0.300	0.270	0.250	0.270	0.061	0.082	0.092	0.099	0.110
Tailings 4	8.700	7.000	6.500	7.100	7.500	0.330	0.300	0.260	0.250	0.240	0.061	0.066	0.074	0.082	0.093

Table AII-6. Dissolved total chromium, copper, iron concentrations during denitrification for multiple AMD-impacted sites

	Chromium (mg/L)					Copper (mg/L)					Iron (mg/L)				
	Time (hours)					Time (hours)					Time (hours)				
	0	24	52	77	149	0	24	52	77	149	0	24	52	77	149
Penn 1	0.026	0.028	0.025	0.025	0.028	3.400	2.300	2.100	1.900	1.600	19.000	39.000	75.000	100.000	220.000
Penn 2	0.026	0.025	0.026	0.029	0.027	3.400	2.400	2.000	1.800	1.600	19.000	36.000	71.000	100.000	210.000
Penn 3	0.026	0.025	0.026	0.024	0.025	3.400	2.400	2.100	1.900	1.600	19.000	35.000	67.000	99.000	210.000
Penn 4	0.026	0.023	0.026	0.023	0.027	3.400	2.400	2.000	1.700	1.700	19.000	34.000	69.000	94.000	220.000
Cinn 1	0.003	0.003	0.003	0.003	0.003	0.190	0.089	0.045	0.031	0.058	0.440	6.900	8.000	8.200	16.000
Cinn 2	0.003	0.006	0.003	0.003	0.003	0.190	0.066	0.061	0.038	0.018	0.440	6.000	9.400	7.700	13.000
Cinn 3	0.003	0.003	0.003	0.003	0.003	0.190	0.074	0.053	0.045	0.041	0.440	6.000	7.500	7.100	12.000
Cinn 4	0.003	0.003	0.003	0.003	0.003	0.190	0.064	0.047	0.031	0.016	0.440	5.500	6.300	7.600	12.000
Snake 1	0.003	0.003	0.003	0.003	0.003	0.020	0.035	0.005	0.120	0.023	1.400	1.400	0.420	2.000	0.990
Snake 2	0.003	0.003	0.003	0.003	0.003	0.020	0.029	0.005	0.005	0.059	1.400	1.400	0.710	0.380	2.100
Snake 3	0.003	0.003	0.003	0.003	0.003	0.020	0.005	0.005	0.005	0.005	1.400	0.710	0.160	0.420	0.570
Snake 4	0.003	0.003	0.003	0.003	0.003	0.020	0.005	0.005	0.005	0.022	1.400	2.300	0.096	0.130	1.600
St. Kevins 1	0.003	0.003	0.003	0.003	0.003	0.170	0.170	0.130	0.130	0.190	0.900	0.750	0.330	2.900	18.000
St. Kevins 2	0.003	0.003	0.003	0.003	0.003	0.170	0.150	0.130	0.100	0.110	0.900	0.500	0.630	1.000	1.100
St. Kevins 3	0.003	0.003	0.003	0.003	0.003	0.170	0.170	0.130	0.120	0.075	0.900	0.750	0.780	1.300	0.640
St. Kevins 4	0.003	0.003	0.003	0.003	0.003	0.170	0.150	0.110	0.084	0.066	0.900	1.000	1.600	1.300	0.470
Tailings 1	0.064	0.058	0.061	0.052	0.052	0.240	0.270	0.260	0.230	0.230	69.000	34.000	21.000	13.000	5.700
Tailings 2	0.064	0.063	0.057	0.053	0.054	0.240	0.270	0.230	0.230	0.190	69.000	34.000	18.000	14.000	4.100
Tailings 3	0.064	0.064	0.060	0.059	0.053	0.240	0.260	0.250	0.240	0.230	69.000	34.000	21.000	14.000	11.000
Tailings 4	0.064	0.058	0.056	0.052	0.051	0.240	0.220	0.170	0.160	0.180	69.000	35.000	20.000	15.000	5.600

Table AII-7. Dissolved total manganese, nickel, and zinc concentrations during denitrification for multiple AMD-impacted sites

	Manganese (mg/L)					Nickel (mg/L)					Zinc (mg/L)				
	Time (hours)					Time (hours)					Time (hours)				
	0	24	52	77	149	0	24	52	77	149	0	24	52	77	149
Penn 1	23.000	25.000	26.000	27.000	29.000	0.160	0.160	0.160	0.160	0.170	31.000	30.000	30.000	30.000	30.000
Penn 2	23.000	26.000	26.000	27.000	29.000	0.160	0.150	0.160	0.150	0.160	31.000	31.000	31.000	30.000	31.000
Penn 3	23.000	26.000	26.000	28.000	29.000	0.160	0.160	0.160	0.150	0.160	31.000	30.000	30.000	30.000	31.000
Penn 4	23.000	24.000	26.000	25.000	30.000	0.160	0.160	0.160	0.150	0.160	31.000	29.000	29.000	26.000	31.000
Cinn 1	2.200	6.900	8.000	8.200	16.000	0.019	0.020	0.018	0.017	0.022	1.600	3.200	2.200	1.800	2.700
Cinn 2	2.200	6.000	9.400	7.700	13.000	0.019	0.019	0.023	0.018	0.018	1.600	2.000	6.200	1.800	2.100
Cinn 3	2.200	6.000	7.500	7.100	12.000	0.019	0.021	0.018	0.015	0.019	1.600	1.800	1.900	1.700	2.100
Cinn 4	2.200	5.500	6.300	7.600	12.000	0.019	0.019	0.015	0.015	0.019	1.600	1.800	1.900	1.800	2.200
Snake 1	1.500	1.000	0.440	0.350	1.000	0.021	0.003	0.003	0.017	0.003	1.600	0.760	0.050	0.440	0.092
Snake 2	1.500	0.490	0.530	0.580	0.910	0.021	0.003	0.003	0.003	0.006	1.600	0.130	0.078	0.081	0.078
Snake 3	1.500	0.480	0.460	0.670	0.840	0.021	0.003	0.003	0.003	0.003	1.600	0.098	0.061	0.200	0.140
Snake 4	1.500	0.480	0.490	0.550	0.880	0.021	0.003	0.003	0.003	0.003	1.600	0.080	0.067	0.063	0.092
St. Kevins 1	2.200	3.400	4.200	6.900	11.000	0.024	0.029	0.026	0.028	0.027	3.900	5.100	4.100	4.800	4.400
St. Kevins 2	2.200	2.700	4.600	6.300	9.700	0.024	0.025	0.026	0.026	0.028	3.900	4.000	4.500	4.600	5.300
St. Kevins 3	2.200	2.500	4.900	6.700	10.000	0.024	0.023	0.028	0.026	0.025	3.900	3.700	4.100	3.900	4.200
St. Kevins 4	2.200	3.800	6.500	8.300	12.000	0.024	0.026	0.025	0.024	0.023	3.900	3.800	3.700	3.700	3.800
Tailings 1	47.000	41.000	41.000	40.000	37.000	0.070	0.069	0.072	0.062	0.071	71.000	64.000	61.000	62.000	53.000
Tailings 2	47.000	43.000	39.000	43.000	40.000	0.070	0.067	0.065	0.069	0.071	71.000	66.000	60.000	62.000	57.000
Tailings 3	47.000	44.000	38.000	39.000	41.000	0.070	0.075	0.073	0.072	0.077	71.000	66.000	56.000	57.000	57.000
Tailings 4	47.000	41.000	37.000	42.000	41.000	0.070	0.071	0.067	0.073	0.074	71.000	61.000	54.000	59.000	54.000

Table AII-8. Nitrate and pH raw data for pH variation experiment

	Sediment Dry Wt. (g)	Nitrate (μM) Time (hours)						pH Time (hours)				
		0	1	26	51	122	166	0	26	51	122	166
pH 2.6 1	54.49	335.64	296.11	285.09	283.12	223.47	31.91	2.60	3.05	3.56	3.77	4.06
pH 2.6 2	47.60	335.64	294.39	287.47	285.23	240.12	103.43	2.60	3.01	3.47	3.69	4.07
pH 2.6 3	48.34	335.64	294.14	290.85	290.24	252.31	152.01	2.59	3.09	3.46	3.68	4.05
pH 2.6 4	49.97	335.64	294.41	285.85	281.61	247.21	127.34	2.60	3.00	3.46	3.69	4.09
pH 3.5 1	47.85	343.95	308.65	279.61	237.31	66.88	0.00	3.55	3.91	4.04	4.24	4.34
pH 3.5 2	50.20	343.95	283.69	275.79	241.05	241.57	0.00	3.56	3.84	4.02	4.22	4.36
pH 3.5 3	51.32	343.95	299.67	277.42	241.07	149.06	33.04	3.57	3.90	4.04	4.25	4.38
pH 3.5 4	50.12	343.95	302.93	278.21	220.16	98.84	0.00	3.59	3.94	4.08	4.30	4.35
Control 1	38.55	336.20	255.99	171.83	31.83	32.64	0.00	4.12	4.19	4.24	4.29	4.32
Control 2	42.51	336.20	216.40	165.21	0.00	0.00	0.00	4.12	4.17	4.27	4.30	4.35
Control 3	44.12	336.20	251.22	155.31	0.00	0.00	0.00	4.12	4.18	4.25	4.32	4.37
Control 4	42.61	336.20	239.83	168.15	0.00	25.88	0.00	4.12	4.2	4.2	4.32	4.37
pH 4.25 1	44.14	342.76	286.87	237.83	175.79	34.81	0.00	4.24	4.31	4.38	5.24	5.41
pH 4.25 2	40.57	342.76	298.10	259.94	178.80	0.00	0.00	4.24	4.34	4.40	5.20	5.42
pH 4.25 3	43.32	342.76	295.53	258.04	180.17	0.00	0.00	4.24	4.33	4.41	5.29	5.49
pH 4.25 4	49.09	342.76	304.11	245.37	181.74	0.00	23.39	4.21	4.28	4.37	5.20	5.48
pH 6.0 1	44.24	334.29	280.52	230.54	129.32	0.00	26.13	6.06	6.48	6.90	7.10	7.16
pH 6.0 2	43.96	334.29	301.67	258.72	164.72	0.00	27.40	6.04	6.53	6.87	7.12	7.21
pH 6.0 3	48.45	334.29	296.78	202.19	88.56	0.00	28.24	6.06	6.59	6.96	7.12	7.18
pH 6.0 4	46.53	334.29	306.47	253.70	166.57	0.00	49.51	6.03	6.47	7.01	7.10	7.19

Table AII-9. Nitrate and pH raw data for electron donor experiment

	Sediment Dry wt. (g)	Nitrate (μM) Time (hours)					pH Time (hours)				
		1	26	50	99	193	1	26	50	99	193
Control 1	31.60003	362.81	305.85	266.46	213.39	69.53	3.05	3.04	3.08	3.10	3.12
Control 2	29.49145	390.32	328.05	297.66	229.61	139.13	3.02	3.03	3.07	3.10	3.12
Control 3	28.86535	352.14	331.01	304.63	242.71	149.22	2.99	3.01	3.07	3.10	3.12
Control 4	32.80186	359.79	321.89	289.78	219.83	126.49	2.97	3.02	3.07	3.10	3.12
Acetate 1	31.6792	353.05	313.70	297.25	268.25	229.73	3.09	3.10	3.09	3.00	3.00
Acetate 2	31.72238	354.46	315.91	292.84	237.81	203.13	3.10	3.12	3.10	2.99	3.00
Acetate 3	31.55686	355.30	314.70	298.22	242.47	191.95	3.10	3.12	3.10	3.00	3.00
Acetate 4	31.31217	360.91	320.41	290.64	245.85	191.18	3.10	3.12	3.10	3.00	3.00
Glucose 1	31.62162	296.32	222.55	153.41	29.88	0.00	3.10	3.11	3.12	3.16	3.40
Glucose 2	30.05997	302.86	238.98	183.16	68.76	0.00	3.10	3.11	3.12	3.15	3.40
Glucose 3	30.40541	311.12	239.18	183.36	64.66	0.00	3.10	3.11	3.12	3.15	3.40
Glucose 4	32.45642	302.46	233.72	170.70	55.59	0.00	3.11	3.12	3.13	3.15	3.40
NOM 1	30.93076	285.40	234.28	200.29	128.25	45.36	3.15	3.17	3.19	3.21	3.27
NOM 2	30.89477	299.72	218.35	181.32	107.82	0.00	3.14	3.16	3.18	3.21	3.27
NOM 3	28.79338	299.52	236.24	197.52	132.28	43.77	3.15	3.17	3.20	3.22	3.27
NOM 4	30.72206	297.45	226.47	174.6	98.13	0.00	3.14	3.15	3.17	3.2	3.27
H2 1	28.50552	247.77	240.80	200.32	164.41	76.34	3.09	3.11	3.12	3.14	3.18
H2 2	29.585	262.07	243.87	210.72	173.26	77.25	3.08	3.10	3.11	3.15	3.18
H2 3	28.80777	250.12	238.31	218.14	158.15	86.27	3.08	3.10	3.11	3.14	3.18
H2 4	30.80122	242.43	231.29	208.01	160.86	81.27	3.08	3.10	3.11	3.14	3.18

Table AII-10. Nitrate concentrations and pH during iron addition denitrification incubations

	Sediment Dry Wt. (g)	Total Fe (mg/L) Time (hours)						Fe^{2+} (mg/L) Time (hours)					
		1	47	122	171	312	456	1	47	122	171	312	456
Control 1	12.52831	291.19	219.87	5.00	0.00	0.00	0.00	4.10	4.51	5.09	4.95	5.03	4.86
Control 2	14.31649	299.84	200.77	5.00	0.00	0.00	0.00	4.10	4.51	5.10	4.98	5.03	4.87
Control 3	13.55209	314.47	208.69	5.00	0.00	0.00	0.00	4.10	4.52	5.08	4.94	5.03	4.84
Control 4	13.95499	295.83	195.34	5.00	0.00	0.00	0.00	4.10	4.52	5.09	4.96	5.03	4.86
50 Fe2 1	12.56694	361.06	293.06	114.14	42.45	0.00	0.00	3.97	4.33	4.46	4.50	5.82	4.90
50 Fe2 2	11.78323	349.29	319.41	125.74	62.59	0.00	0.00	3.97	4.34	4.45	4.49	5.82	4.90
50 Fe2 3	11.8191	368.86	314.96	108.05	61.58	0.00	0.00	3.97	4.34	4.45	4.50	5.82	4.90
50 Fe2 4	12.65248	372.41	293.71	168.15	61.75	0.00	0.00	3.97	4.32	4.46	4.50	5.82	4.90
200 Fe2 1	11.91293	340.20	279.46	88.07	0.00	0.00	0.00	4.00	4.20	4.30	4.30	4.60	4.60
200 Fe2 2	11.98468	351.68	285.91	78.38	0.00	0.00	0.00	4.00	4.20	4.31	4.29	4.60	4.60
200 Fe2 3	12.69388	353.99	292.16	100.99	0.00	0.00	0.00	4.00	4.20	4.32	4.29	4.60	4.60
200 Fe2 4	12.77666	344.70	285.00	95.94	0.00	0.00	0.00	4.00	4.20	4.31	4.30	4.60	4.60
50 Fe3 1	12.98087	357.27	323.37	204.82	33.99	0.00	0.00	3.66	3.78	4.41	4.52	4.70	4.80
50 Fe3 2	12.57522	391.33	330.75	206.53	165.87	0.00	0.00	3.67	3.77	4.40	4.51	4.70	4.80
50 Fe3 3	13.52726	352.24	320.64	204.07	53.62	0.00	0.00	3.66	3.78	4.41	4.52	4.70	4.80
50 Fe3 4	12.43448	381.64	337.25	121.26	65.42	0.00	0.00	3.67	3.77	4.40	4.51	4.70	4.80
200 Fe3 1	12.36549	320.57	309.56	233.30	185.69	78.37	0.00	3.17	4.29	4.04	4.13	4.38	4.60
200 Fe3 2	12.7877	321.87	310.34	213.93	194.06	98.79	0.00	3.17	4.29	4.05	4.14	4.38	4.60
200 Fe3 3	12.41792	313.41	335.35	254.83	182.63	164.38	72.54	3.17	4.29	4.06	4.13	4.38	4.60
200 Fe3 4	13.2927	317.68	305.66	237.12	159.20	54.80	0.00	3.17	4.29	4.05	4.13	4.38	4.60

Table AII-11. Total iron and Fe^{2+} concentrations during iron addition denitrification incubations

	Total Fe (mg/L) Time (hours)						Fe^{2+} (mg/L) Time (hours)					
	1	47	122	171	312	456	1	47	122	171	312	456
Control 1	23.15	39.26	44.782	40.3775	43.1165	40.2655	22.587	38.99	38.142	39.989	41.681	39.911
Control 2	23.53	40.23	40.157	53.262	49.2555	53.444	23.236	39.35	39.125	52.348	47.526	53.319
Control 3	25.70	36.24	52.784	40.942	49.66	47.1395	25.317	36.07	51.43	40.6105	47.921	45.236
Control 4	25.20	35.23	41.632	40.6525	46.9655	37.46	23.168	34.64	38.176	40.5885	45.2355	37.164
50 Fe2 1	71.87	73.61	62.729	45.6445	88.639	21.04	70.336	69.99	52.669	45.327	86.300	20.809
50 Fe2 2	68.28	74.85	74.083	77.2285	87.265	90.2095	67.938	74.20	73.921	57.055	86.133	89.9995
50 Fe2 3	73.01	81.93	61.636	77.337	89.2795	86.734	71.727	80.32	61.062	75.613	87.457	86.6155
50 Fe2 4	71.54	82.32	81.777	86.3795	97.106	102.87	65.264	82.22	81.366	85.5205	96.2195	101.315
200 Fe2 1	312.83	297.64	269.14	245.605	264.87	246.985	306.06	286.47	265.72	240.405	259.80	244.895
200 Fe2 2	327.14	316.53	284.18	273.935	280.29	269.75	326.51	310.03	282.27	272.76	277.25	265.245
200 Fe2 3	328.28	310.09	292.49	273.035	305.36	285.335	323.59	307.50	290.38	269.685	297.41	283.755
200 Fe2 4	323.28	310.61	279.701	267.605	282.67	245.16	320.38	305.84	279.49	259.835	276.63	242.61
50 Fe3 1	21.18	41.49	35.847	36.4445	61.734	46.9125	21.097	38.26	35.052	34.886	61.0505	46.868
50 Fe3 2	22.33	40.01	6.491	38.2145	65.3395	43.984	21.35	37.86	3.2219	33.0975	64.434	42.467
50 Fe3 3	25.53	36.92	44.048	39.1395	63.982	66.0375	25.22	34.31	40.741	38.872	61.5235	65.8695
50 Fe3 4	26.17	41.67	39.885	38.783	64.273	39.7517	25.462	40.17	39.405	37.6585	62.9335	39.316
200 Fe3 1	39.64	64.67	89.510	90.277	77.767	92.602	34.267	61.60	88.243	88.9385	71.590	90.810
200 Fe3 2	40.24	69.57	89.146	96.616	78.275	101.57	37.229	67.15	86.23	92.22275	74.105	97.682
200 Fe3 3	39.85	67.99	91.963	89.26	72.736	97.5665	36.286	66.65	87.6678	88.3245	74.434	96.151
200 Fe3 4	43.37	70.61	89.654	92.131	75.28	92.0985	39.976	69.93	88.516	90.255	71.6455	90.7395

Appendix III: Raw data from Chapter 5

Table AIII-1. Snowcover Nitrate (μM) for Denitrification Rate Constant Calculations

Flask	Sediment Dry Weight (mg)	Time (hours)				
		0	24	72	168	336
Deer 1	40.95	345.1	317.2	5.00	31.00	5.00
Deer 2	38.52	349.7	301.1	5.00	31.00	30.00
Deer 3	41.58	351.3	317.6	5.00	31.00	5.00
Deer 4	34.76	294.1	287.9	5.00	NA	NA
Snake 1	66.67	369.0	305.5	38.43	40.04	5.00
Snake 2	70.07	380.9	320.1	5.00	39.76	5.00
Snake 3	67.03	386.0	322.1	5.00	39.07	5.00
Snake 4	65.83	381.2	312.7	5.00	38.73	5.00
Penn 1	38.83	305.1	279.8	220.4	135.9	5.00
Penn 2	33.23	325.5	302.9	240.0	153.6	26.63
Penn 3	32.77	346.9	304.1	243.2	166.8	55.39
Penn 4	34.79	319.7	297.3	242.4	160.3	44.71
Peru 1	33.29	380.8	197.0	5.00	5.00	38.88
Peru 2	40.80	289.4	215.2	75.00	5.00	39.15
Peru 3	41.27	299.7	232.6	85.00	38.64	38.89
Peru 4	40.78	298.4	226.7	94.33	5.00	38.75

Table AIII-2. Snowcover pH and Sulfate Raw Data

Flask	pH					Sulfate (M)				
	Time (hrs)					Time (hrs)				
	0	24	72	168	336	0	24	72	168	336
Deer 1	5.99	6.26	6.73	6.72	6.73	239.5	266.2	238.6	258.3	463.5
Deer 2	6.08	6.28	6.72	6.73	6.72	233.6	255.8	251.0	348.9	391.1
Deer 3	6.10	6.29	6.71	6.72	6.73	234.4	248.8	245.8	284.1	361.3
Deer 4	6.10	6.30	6.72	NA	NA	178.5	239.3	278.0	NA	NA
Snake 1	5.32	5.58	5.72	6.00	6.21	1274	1362	1622	1931	3136
Snake 2	5.31	5.59	5.72	6.00	6.21	1307	1410	1769	2277	3522
Snake 3	5.30	5.60	5.73	6.00	6.20	1307	1408	1698	1886	3904
Snake 4	5.30	5.59	5.73	6.00	6.21	1303	1387	1687	2048	3246
Penn 1	3.17	3.21	3.24	3.27	3.30	7588	7755	9315	11800	17020
Penn 2	3.17	3.20	3.23	3.27	3.31	7854	7997	9228	11260	14880
Penn 3	3.17	3.21	3.24	3.28	3.31	7990	8159	9654	12030	16280
Penn 4	3.18	3.20	3.24	3.27	3.31	7778	8137	9814	11960	15710
Peru 1	4.62	4.67	4.73	4.88	4.92	1024	1147	1520	2087	3871
Peru 2	4.62	4.68	4.73	4.88	4.92	1072	1180	1541	1869	2611
Peru 3	4.62	4.68	4.73	4.88	4.92	1054	1147	1380	1705	2372
Peru 4	4.62	4.68	4.73	4.88	4.92	1079	1196	1424	1737	2301

Table AIII-3. Snowmelt Nitrate (μM) for Denitrification Rate Constant Calculations

Flask	Sediment Dry Weight (mg)	Time (hours)				
		0	24	72	168	336
Deer 1	26.65	293.3	108.4	17.76	19.55	NA
Deer 2	31.29	283.0	102.3	17.25	20.54	23.24
Deer 3	25.59	310.0	141.0	18.15	19.67	19.44
Deer 4	27.78	304.0	110.2	16.18	19.66	23.02
Snake 1	40.60	285.1	277.7	195.2	165.3	38.50
Snake 2	44.27	282.0	267.4	190.3	152.6	5.00
Snake 3	41.14	286.1	260.6	196.5	158.7	39.33
Snake 4	39.91	290.8	274.0	191.8	154.0	38.63
Penn 1	24.55	300.3	273.9	149.0	91.89	5.00
Penn 2	23.67	313.0	290.9	166.2	112.8	5.00
Penn 3	26.58	313.7	267.0	128.0	67.35	5.00
Penn 4	24.01	311.1	283.1	183.3	137.7	5.00
Peru 1	39.55	391.9	384.7	372.5	347.1	264.1
Peru 2	40.70	392.3	380.3	349.3	338.3	248.1
Peru 3	42.10	392.4	377.2	359.3	321.6	232.7
Peru 4	41.20	391.5	375.5	381.8	379.8	324.4

Table AIII-4. Snowmelt pH and Sulfate Raw Data

Flask	pH					Sulfate (□M)				
	Time (hrs)					Time (hrs)				
	0	24	72	168	336	0	24	72	168	336
Deer 1	5.99	603	6.36	6.49	NA	493.3	601.3	694.6	844.1	NA
Deer 2	5.97	6.03	6.32	6.50	6.51	519.4	836.9	640.4	902.3	1025
Deer 3	6.03	6.05	6.34	6.49	6.50	495.5	580.5	743.2	903.1	1012
Deer 4	6.04	6.06	6.33	6.49	6.50	511.3	587.8	787.2	906.6	1053
Snake 1	3.95	3.96	4.20	4.32	4.45	423.2	496.0	734.2	916.9	1349
Snake 2	3.93	3.97	4.19	4.33	4.47	432.0	483.2	816.8	975.2	1520
Snake 3	3.95	3.96	4.18	4.33	4.45	426.5	472.7	817.9	972.2	1465
Snake 4	3.95	3.97	4.19	4.32	4.46	427.1	481.4	784.3	959.6	1437
Penn 1	3.56	3.65	3.71	3.76	3.88	3688	4383	7212	8429	13150
Penn 2	3.56	3.65	3.72	3.76	3.89	3903	4100	6472	7767	11840
Penn 3	3.53	3.64	3.71	3.76	3.89	3901	4577	7397	8170	13380
Penn 4	3.48	3.54	3.62	3.78	3.89	3874	4147	6501	7526	11460
Peru 1	4.63	4.65	4.82	4.93	5.03	423.0	490.0	659.3	741.4	978.
Peru 2	4.60	4.63	4.81	4.93	5.04	430.4	479.6	636.3	730.8	991.4
Peru 3	4.60	4.63	4.82	4.92	5.03	434.9	469.9	658.8	716.0	998.7
Peru 4	4.61	4.64	4.82	4.93	5.03	432.8	487.9	654.8	732.2	1033

Table AIII-5. Summer Nitrate (μM) for Denitrification Rate Constant Calculations

Flask	Sediment Dry Weight (mg)	Time (hours)				
		0	24	72	168	336
Deer 1	39.69	290.7	178.0	42.07	38.93	42.24
Deer 2	37.66	293.4	158.8	41.23	44.46	42.41
Deer 3	39.18	294.0	164.7	40.69	42.96	44.96
Deer 4	36.99	289.8	180.7	41.33	44.08	44.03
Snake 1	55.04	333.6	318.2	314.0	253.7	234.0
Snake 2	48.87	332.8	328.0	318.2	288.2	271.3
Snake 3	47.62	336.3	332.7	312.2	304.7	266.5
Snake 4	52.62	331.9	329.4	310.9	269.0	241.9
Penn 1	29.21	271.3	250.1	214.4	156.6	76.33
Penn 2	33.82	279.4	267.3	232.3	188.7	124.5
Penn 3	37.92	268.1	255.1	229.9	173.9	92.05
Penn 4	34.99	287.7	271.2	238.0	195.1	139.5
Peru 1	51.66	345.2	336.3	321.3	266.6	151.2
Peru 2	49.51	357.8	346.1	326.8	256.4	159.0
Peru 3	47.53	359.1	350.8	334.5	255.8	140.7
Peru 4	50.37	361.0	352.0	328.3	295.8	218.8

Table AIII-6. Summer pH and Sulfate Raw Data

Flask	pH					Sulfate (μM)				
	Time (hrs)					Time (hrs)				
	0	24	72	168	336	0	24	72	168	336
Deer 1	6.16	6.28	6.43	6.48	6.49	280.7	325.0	369.4	442.4	622.2
Deer 2	6.25	6.31	6.43	6.49	6.48	294.6	365.3	429.8	516.6	635.0
Deer 3	6.29	6.30	6.44	6.48	6.49	293.5	365.8	460.9	164.5	608.8
Deer 4	6.33	6.32	6.44	6.48	6.49	274.0	363.9	436.8	529.5	554.0
Snake 1	4.00	4.14	4.37	4.52	4.58	652.7	648.1	789.0	1047	1451
Snake 2	4.08	4.12	4.36	4.51	4.59	646.4	703.0	805.1	1047	2070
Snake 3	4.09	4.15	4.37	4.52	4.58	643.7	663.9	810.3	1014	1470
Snake 4	4.12	4.10	4.38	4.52	4.58	641.9	668.2	832.4	953.7	1491
Penn 1	2.93	2.96	3.01	3.08	3.21	13340	13060	14080	15540	18570
Penn 2	2.99	3.02	3.03	3.07	3.20	13410	13320	14020	15750	18460
Penn 3	2.95	2.99	3.05	3.07	3.21	13600	13260	14250	16190	19700
Penn 4	2.95	2.99	3.05	3.08	3.21	14290	13980	14610	16430	19100
Peru 1	4.11	4.17	4.22	4.30	4.40	474.0	564.8	728.5	987.1	1367
Peru 2	4.12	4.16	4.23	4.29	4.39	487.3	585.1	753.3	963.5	1277
Peru 3	4.11	4.18	4.23	4.29	4.40	478.3	587.8	783.0	1110	1577
Peru 4	4.10	4.16	4.23	4.29	4.40	480.9	583.6	756.8	1040	1669

APPENDIX IV: Microbial Diversity Raw Data

Table A-IV 1. Deer Creek Snowcover: ARB Divisions

ARB Name	RFLP type	Number of RFLPs	ARB Division	ARB Subdivision		Bases
BDC1_A01	1	1	Proteobacteria	Beta	Burkholderiales	775
BDC1_B01	3	1	Gemmimonas	environmental		813
BDC1_B02	4	1	Proteobacteria	Alpha	DA122 soil group	788
BDC1_C02	6	1	Proteobacteria	Beta	Burkholderiales	790
BDC1_D01	7	1	Proteobacteria	Beta	Burkholderiales	787
BDC1_D02	8	1	Actinobacteria	Propionibacterineae	environmental	772
BDC1_E01	9	1	Actinobacteria	Acidimicrobidae	acidimicrobium	792
BDC1_E02	10	1	Proteobacteria	Alpha	environmental	777
BDC1_F02	12	1	Proteobacteria	Alpha	Sphingomonadales	768
BDC1_G01	13	1	Proteobacteria	Delta	Desulfuromonadales	776
BDC1_G02	14	1	Proteobacteria	Alpha	Rhizobiales	771
BDC1_H01	15	1	Proteobacteria	Beta	Burkholderiales	781
BDC1_H02	16	1	Actinobacteria	Acidimicrobidae	acidimicrobium	763
BDC1_A03	17	1	Bacteroidetes	Saprospiraceae	environmental	764
BDC1_A04	18	1	Proteobacteria	Beta	Burkholderiales	778
BDC1_B03	19	1	Acidobacteria	Acidobacteria-4	environmental	796
BDC1_B04	20	1	termite gut 1	environmental		781
BDC1_D03	23	1	Proteobacteria	Alpha	environmental	764
BDC1_E04	26	1	Proteobacteria	Alpha	Sphingomonadales	633
BDC1_F03	27	1	Deinococcoccus	environmental		752
BDC1_F04	28	1	Gemmimonas	environmental		786
BDC1_H03	31	1	Proteobacteria	Beta	Burkholderiales	768
BDC1_A06	34	1	Proteobacteria	Alpha	environmental	827
BDC1_B05	35	1	Chloroflexi	Chloroflexi-1	environmental	753
BDC1_B06	36	1	Proteobacteria	Gamma	Environ. clone group	773
BDC1_C05	37	1	Bacteroidetes	Saprospiraceae	environmental	755
BDC1_C06	38	1	Proteobacteria	Alpha	Rhizobiales	782
BDC1_D05	39	1	Proteobacteria	Beta	Burkholderiales	767
BDC1_D06	40	1	Proteobacteria	Beta	Burkholderiales	741
BDC1_E05	41	1	Actinobacteria	Acidimicrobidae	acidimicrobium	738
BDC1_E06	42	1	Proteobacteria	Beta	Burkholderiales	775
BDC1_F05	43	1	Proteobacteria	Alpha	Rhodobacterales	739
BDC1_F06	44	1	Gemmimonas	environmental		801
BDC1_G05	45	1	Actinobacteria	Acidimicrobidae	acidimicrobium	706
BDC1_G06	46	1	Acidobacteria	Acidobacteria-6	environmental	782
BDC1_H05	47	1	Acidobacteria	Acidobacteria-4	environmental	755
BDC1_H06	48	1	Proteobacteria	Alpha	environmental	776
BDC1_A07	49	1	Proteobacteria	Beta	Burkholderiales	750
BDC1_A08	50	1	Proteobacteria	Alpha	Sphingomonadales	753
BDC1_B07	51	1	Actinobacteria	Acidimicrobidae	acidimicrobium	780
BDC1_B08	52	1	Planctomycetes	Pirellula	environmental	711

Table A-IV 1. Deer Creek Snowcover: ARB Divisions, continued

ARB Name	RFLP type	Number of RFLPs	ARB Division	ARB Subdivision		Bases
BDC1_C07	53	1	Chloroflexi	Chloroflexi-1	environmental	734
BDC1_C08	54	1	Bacteroidetes	Saprospiraceae	environmental	764
BDC1_D07	55	1	Acidobacteria	Acidobacteria-6	environmental	731
BDC1_D08	56	1	Proteobacteria	Beta	environmental	758
BDC1_E07	57	1	Proteobacteria	Beta	environmental	747
BDC1_E08	58	1	Proteobacteria	Beta	Burkholderiales	748
BDC1_F07	59	1	Planctomycetes	Pirellula	environmental	759
BDC1_F08	60	1	Proteobacteria	Beta	Burkholderiales	778
BDC1_G07	61	1	Proteobacteria	Delta	Myxococcales	709
BDC1_G08	62	1	Proteobacteria	Alpha	DA122 soil group	712
BDC1_H07	63	1	Proteobacteria	Beta	environmental	751
BDC1_H08	64	1	Proteobacteria	Alpha	Hyphomicrobiaceae2	752
BDC1_A09	65	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	785
BDC1_A10	66	1	Proteobacteria	Beta	Burkholderiales	757
BDC1_B09	67	1	Proteobacteria	Gamma	Environ. clone group	697
BDC1_B10	68	1	Proteobacteria	Beta	Burkholderiales	762
BDC1_C09	69	1	Verrucomicrobia	VER-3	environmental	807
BDC1_D09	71	1	Actinobacteria	Acidimicrobidae	acidimicrobium	750
BDC1_E09	73	1	Actinobacteria	Propionibacterineae	environmental	761
BDC1_E10	74	1	Acidobacteria	Acidobacteria-4	environmental	740
BDC1_F09	75	1	Bacteroidetes	Saprospiraceae	environmental	760
BDC1_F10	76	1	Nitrospira	ferronmanganous		762
BDC1_G09	77	1	Gemmimonas	environmental		762
BDC1_H09	79	1	Proteobacteria	Beta	Burkholderiales	748
BDC1_H10	80	1	Actinobacteria	Rubrobacteridae	MC4	760
BDC1_A12	82	1	Proteobacteria	Gamma	Environ. clone group	795
BDC1_B11	83	1	Chloroflexi	Chloroflexi-1	Chloroflexi 1a	744
BDC1_C11	85	1	Proteobacteria	Beta	environmental	742
BDC1_D11	87	1	Proteobacteria	Alpha	environmental	760
BDC1_D12	88	1	Proteobacteria	Beta	environmental	778
BDC1_E11	89	1	Acidobacteria	Acidobacteria-6	environmental	743
BDC1_E12	90	1	Chlamydiae	endosymbionts of Acanthamoeba sp.		772
BDC1_F11	91	1	Chloroflexi	Chloroflexi-1	environmental	738
BDC1_F12	92	1	Planctomycetes	Pirellula	environmental	771
BDC1_G11	93	1	Proteobacteria	Alpha	environmental	768
BDC1_G12	94	1	Bacteroidetes	Saprospiraceae	environmental	796
BDC1_H11	95	1	Bacteroidetes	Saprospiraceae	environmental	812

Table A-IV 2. Deer Creek Snowcover: Best Blast Matches

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BDC1_A01	AB008503	1108	93	Ultramicrobacterium str. MY14 gene for 16S rRNA, partial
BDC1_B01	AF432607	878	90	Uncultured bacterium clone NOS757WL 16S rRNA, partial
BDC1_B02	AF523949	1332	96	Uncultured bacterium clone FW91 16S rRNA, partial
BDC1_C02	AB008503	1116	93	Ultramicrobacterium str. MY14 gene for 16S rRNA, partial
BDC1_D01	AF543312	1219	94	Herbaspirillum lusitanum 16S rRNA, complete sequence
BDC1_D02	AB097215	1522	99	Propionibacterium acnes gene for 16S ribosomal RNA, complete sequence
BDC1_E01	AY093455	1174	93	Uncultured bacterium clone MB-A2-100 16S ribosomal RNA, partial
BDC1_E02	AJ532709	1509	99	Uncultured alpha proteobacterium partial 16S rRNA clone
BDC1_F02	AJ427917	1074	93	Sphingomonas sp. JS1 16S rRNA gene
BDC1_G01	AY013647	1467	98	Uncultured Banisveld landfill bacterium BVB45 16S ribosomal RNA
BDC1_G02	AJ271900	1322	96	Mesorhizobium sp. partial 16S rRNA strain rob23
BDC1_H01	AF078755	1334	96	Aquaspirillum psychrophilum 16S rRNA, partial
BDC1_H02	AF328189	1348	97	Uncultured bacterium clone KS48 16S rRNA, partial
BDC1_A03	AF314430	993	91	Uncultured bacterium PHOS-HE31 16S rRNA, partial
BDC1_A04	AJ505858	1344	96	Comamonadaceae bacterium PIV-8-2 partial 16S rRNA strain
BDC1_B03	Z95709	1047	91	Bacterial species 16S rRNA gene (clone 11-25)
BDC1_B04	AJ001605	77	91	Wolbachia sp. (from L. oceanica) 16S rRNA partial
BDC1_D03	AF277479	299	88	Uncultured alpha proteobacterium SIC.42340 16S rRNA,
BDC1_E04	AY145547	1072	96	Alpha proteobacterium AP-9-1 16S rRNA, partial
BDC1_F03	AB022911	553	87	Deinococcus sp. MBIC3950 gene for 16S rRNA, partial
BDC1_F04	AF545649	1407	97	Uncultured candidate division BD bacterium clone BDfull1 16S
BDC1_H03	AF078755	1292	96	Aquaspirillum psychrophilum 16S rRNA, partial
BDC1_A06	AB029352	159	89	Angiopteris lygodiifolia mitochondrial gene for 18S rRNA
BDC1_B05	AJ532729	868	89	Uncultured bacterium partial 16S rRNA clone JG34-KF-221
BDC1_B06	AJ292676	1086	93	uncultured eubacterium WD2124 partial 16S rRNA clone WD2124
BDC1_C05	AF527580	1124	93	Uncultured bacterium clone LPB08 16S rRNA, partial
BDC1_C06	AJ271900	1390	97	Mesorhizobium sp. partial 16S rRNA strain rob23
BDC1_B09	AJ318105	874	92	Uncultured bacterium 16S rRNA clone BIai14
BDC1_B10	AJ505858	1320	96	Comamonadaceae bacterium PIV-8-2 partial 16S rRNA strain
BDC1_D05	AB074523	1441	98	Aquaspirillum arcticum gene for 16S rRNA, partial
BDC1_D06	AF078755	1265	96	Aquaspirillum psychrophilum 16S rRNA, partial
BDC1_E05	AF543499	1013	92	Uncultured bacterium clone ASL8 16S rRNA, partial
BDC1_E06	AF414585	1013	91	Uncultured bacterium clone P3OU-26 16S rRNA, partial
BDC1_F05	AY056831	684	88	Rhizobium sp. CA8561 16S rRNA, partial
BDC1_F06	AF432607	878	90	Uncultured bacterium clone NOS757WL 16S rRNA, partial
BDC1_G05	AB003935	1199	96	Kineosporia rhamnosa gene for 16S rRNA, partial, strain:
BDC1_G06	Z95710	1423	98	Bacterial species 16S rRNA gene (clone 32-10)
BDC1_H05	Z95709	975	91	Bacterial species 16S rRNA gene (clone 11-25)
BDC1_H06	AJ519651	1507	99	Uncultured alpha proteobacterium partial 16S rRNA clone
BDC1_A07	AF078755	1289	96	Aquaspirillum psychrophilum 16S rRNA, partial
BDC1_A08	AJ427917	.1160	94	Sphingomonas sp. JS1 16S rRNA gene
BDC1_B07	AJ532727	1435	98	Uncultured bacterium partial 16S rRNA clone JG34-KF-316
BDC1_B08	AF424497	1094	95	Uncultured planctomycete MERTZ_21CM_287 16S rRNA,
BDC1_C07	AJ532729	1296	97	Uncultured bacterium partial 16S rRNA clone JG34-KF-221

Table A-IV 2. Deer Creek Snowcover: Best Blast Matches, cotinued

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BDC1_C08	AY124340	1031	92	Bacterium CS57 16S rRNA, partial
BDC1_D07	Z95727	1142	95	Bacterial species 16S rRNA gene (clone ii3-7)
BDC1_D08	AF272422	1049	92	Nitrosomonas oligotropha 16S rRNA, partial
BDC1_E07	AJ519656	1045	92	Uncultured beta proteobacterium partial 16S rRNA clone
BDC1_E08	AF236012	1308	97	Beta proteobacterium A0837 16S rRNA, partial
BDC1_F07	AJ252628	1417	98	Agricultural soil bacterium clone SC-I-28, 16S rRNA gene (partial)
BDC1_F08	AF078755	1360	97	Aquaspirillum psychrophilum 16S rRNA, partial
BDC1_G07	AF424218	1199	96	Uncultured delta proteobacterium MERTZ_2CM_249 16S ribosomal RNA
BDC1_G08	AF292996	1350	99	Uncultured Green Bay ferromanganous micronodule bacterium MNF4 16S
BDC1_H07	AJ007650	1411	98	Uncultured proteobacterium 16S rRNA clone 1405-9, partial
BDC1_H08	AY159133	765	98	Uncultured bacterium MF_clone_16 16S rRNA, partial
BDC1_A09	AF514854	1427	97	Haslea salstonica 16S rRNA, partial;
BDC1_A10	X97070	1326	97	L.cholodnii 16S rRNA gene
BDC1_C09	U62845	1181	93	Unidentified eubacterium RB24 16S rRNA, partial
BDC1_D09	AB097215	1471	99	Propionibacterium acnes gene for 16S ribosomal RNA, complete sequence
BDC1_E09	AB097215	1509	100	Propionibacterium acnes gene for 16S ribosomal RNA, complete sequence
BDC1_E10	AY094624	1005	92	Uncultured bacterium clone SM1H07 16S rRNA, partial
BDC1_F09	AF314430	1342	97	Uncultured bacterium PHOS-HE31 16S rRNA, partial
BDC1_F10	AF293010	1463	99	Uncultured Green Bay ferromanganous micronodule bacterium MNC2 16S
BDC1_G09	AF432607	615	97	Uncultured bacterium clone NOS757WL 16S rRNA,
BDC1_H09	AF035054	1380	98	Beta proteobacterium B8 16S rRNA, partial
BDC1_H10	AF498707	763	89	Bacterium Ellin325 16S rRNA, partial
BDC1_A12	AF523889	1098	92	Uncultured bacterium clone RCP1-57 16S rRNA, partial
BDC1_B11	AJ307949	1437	99	uncultured bacterium mRNA for 16S ribosomal RNA, clone sipK52
BDC1_C11	AJ519656	1003	92	Uncultured beta proteobacterium partial 16S rRNA clone
BDC1_D11	U62846	1108	93	Unidentified eubacterium RB25 16S rRNA, partial
BDC1_D12	AJ519656	1051	92	Uncultured beta proteobacterium partial 16S rRNA clone
BDC1_E11	AJ532717	1342	97	Uncultured bacterium partial 16S rRNA clone JG34-KF-27
BDC1_E12	AF308693	1251	96	Uncultured bacterium corvenA4 16S rRNA, partial
BDC1_F11	AB067647	765	88	Anaerobic filamentous bacterium STL-6-O1 gene for 16S rRNA, partial
BDC1_F12	AJ290170	1445	98	Uncultured planctomycete partial 16S rRNA clone DSP04
BDC1_G11	AJ532709	1411	98	Uncultured alpha proteobacterium partial 16S rRNA clone
BDC1_G12	AF527580	1174	93	Uncultured bacterium clone LPB08 16S rRNA, partial
BDC1_H11	AJ290025	1330	96	Uncultured bacterium GKS2-106 16S rRNA gene

Table A-IV 3. Deer Creek Snowcover: Best BLAST Matches for sequences not 'tree-ed' in ARB and partials

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BDC1_H12	AJ534617	1437	98	Uncultured alpha proteobacterium partial 16S rRNA clone
BDC1_H04	AJ532727	1437	98	Uncultured bacterium partial 16S rRNA clone JG34-KF-316
BDC1_A02	AJ532709	1259	95	Uncultured alpha proteobacterium partial 16S rRNA clone
BDC1_C01	AF078755	1366	96	Aquaspirillum psychrophilum 16S rRNA, partial
BDC1_C03	AB097215	1556	99	Propionibacterium acnes gene for 16S rRNA, complete sequence
BDC1_C04	AB097215	1586	99	Propionibacterium acnes gene for 16S rRNA, complete sequence
BDC1_D04	AF234724	1183	94	Uncultured sludge bacterium A39 16S rRNA, partial
BDC1_E03	AF523912	940	90	Uncultured bacterium clone RCP1-37 16S rRNA, partial
BDC1_G03	AB022911	769	87	Deinococcus sp. MBIC3950 gene for 16S rRNA, partial
BDC1_G04	AB003935	1394	96	Kineosporia rhamnosa gene for 16S rRNA, partial, strain:
BDC1_A05	AF499401	238	87	Uncultured bacterium clone JCL1-36 16S rRNA, partial
BDC1_C10	AJ401106	884	95	Uncultured verrucomicrobium DEV006 partial 16S rRNA clone
BDC1_D10	AY094624	1128	96	Uncultured bacterium clone SM1H07 16S rRNA, partial
Partials				
BDC1_A11	AB074523	1126	97	Aquaspirillum arcticum gene for 16S rRNA, partial
BDC1_C12	AB087364	569	95	Uncultured bacterium gene for 16S rRNA, partial, clone:F9
BDC1_F01	AF005747	61	97	Uncultured eubacterium H1.2.f 16S rRNA, partial

Table A-IV 4. Deer Creek Snowmelt: Best BLAST Matches for sequences not 'tree-ed' in ARB and partials

ARB Name	RFLP type	Bit Score	% ID	Best Blast Match
BDC2_D02	8	1513	98	Uncultured bacterium partial 16S rRNA clone JG34-KF-135
BDC2_G02	14	1540	98	Uncultured bacterium L3-17C4 16S rRNA, partial
BDC2_A03	17	862	88	Beggiatoa alba 16S ribosomal RNA (16S rRNA) gene, partial
BDC2_B03	19	1037	91	Uncultured planctomycete MERTZ_21CM_155 16S rRNA,
BDC2_C03	21	1370	96	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BDC2_C04	22	549	93	Uncultured archaeon CLEAR-24 16S rRNA, partial
BDC2_D03	23	1461	98	Uncultured bacterium clone HTB10 16S rRNA, partial
BDC2_D04	24	1392	96	Bacterial species 16S rRNA gene (clone iii1-15)
BDC2_E03	25	1263	94	Uncultured Holophaga sp. partial 16S rRNA clone JG37-AG-144
BDC2_B06	36	1193	93	Uncultured bacterium clone GOUTB18 16S rRNA, partial
BDC2_C06	38	1517	98	Beta proteobacterium BP-5 16S rRNA, partial
BDC2_D06	40	1457	97	Uncultured alpha proteobacterium partial 16S rRNA clone
BDC2_E06	42	1499	98	Uncultured bacterium clone FW48 16S rRNA, partial
BDC2_H05	47	710	99	Uncultured bacterium 124 16S rRNA, partial
BDC2_A08	50	622	92	Uncultured bacterium 16S rRNA clone TK97
BDC2_G07	61	1221	94	P.manganicum 16S rDNA
BDC2_A09	65	1245	97	Uncultured Banisveld landfill bacterium BVA77 16S rRNA,
BDC2_E11	89	694	92	Uncultured bacterium clone FW97 16S rRNA, partial
BDC2_G12	94	1437	98	Uncultured alpha proteobacterium partial 16S rRNA clone
Partial				
BDC2_D08	56	361	95	Uncultured beta proteobacterium isolate HS-58 16S ribosomal RNA

Table A-IV 5. Deer Creek Snowmelt: ARB Divisions

ARB Name	RFLP type	Number of RFLPs	ARB division	ARB subdivision		Bases
BDC2_A01	1	1	Actinobacteria	Microbacteriaceae	environmental	805
BDC2_A02	2	1	Proteobacteria	Alpha	environmental	809
BDC2_B01	3	1	Actinobacteria	Acidimicrobidae	acidimicrobium	823
BDC2_B02	4	1	Acidobacteria	Acidobacteria-3	environmental	822
BDC2_C01	5	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	801
BDC2_C02	6	1	Proteobacteria	Alpha	environmental	769
BDC2_D01	7	1	Proteobacteria	Beta	Burkholderiales	825
BDC2_E01	9	1	Proteobacteria	Alpha	environmental	837
BDC2_E02	10	1	Proteobacteria	Gamma	Environ. clone group	821
BDC2_F01	11	1	Actinobacteria	Intrasporangiaaceae	environmental	808
BDC2_F02	12	1	Bacteroidetes	Flexibacteraceae	environmental	759
BDC2_G01	13	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	795
BDC2_H01	15	1	Bacteroidetes	Flavobacteriales	Sporocytophaga	765
BDC2_H02	16	1	Actinobacteria	Acidimicrobidae	acidimicrobium	845
BDC2_A04	18	1	Acidobacteria	Acidobacteria-4	environmental	751
BDC2_B04	20	1	TM7	TM7-1		795
BDC2_E04	26	1	Proteobacteria	Alpha	environmental	816
BDC2_F03	27	1	Acidobacteria	Acidobacteria-3	environmental	756
BDC2_F04	28	1	Proteobacteria	Beta	Burkholderiales	809
BDC2_G03	29	1	Proteobacteria	Beta	environmental	814
BDC2_G04	30	1	Verrucomicrobia	VER-3	environmental	804
BDC2_H03	31	1	Firmicutes	Chlostridium et al	caloramator et al	573
BDC2_H04	32	1	Proteobacteria	Delta	Desulfuromonadales	802
BDC2_A05	33	1	Proteobacteria	Alpha	environmental	822
BDC2_A06	34	1	Proteobacteria	Gamma	γ-proteobacteria A	785
BDC2_B05	35	1	Proteobacteria	Beta	Burkholderiales	817
BDC2_C05	37	1	Proteobacteria	Alpha	Rhodobacterales	803
BDC2_D05	39	1	Proteobacteria	Gamma	environmental	805
BDC2_E05	41	1	Proteobacteria	Alpha	environmental	810
BDC2_F05	43	1	Acidobacteria	Acidobacteria-4	environmental	798
BDC2_F06	44	1	Cyanobacteria	chloroplasts	chlorella chloroplast	832
BDC2_G05	45	1	Acidobacteria	Acidobacteria-6	environmental	814
BDC2_G06	46	1	Nitrospira	Ferromanganous micronodule		780
BDC2_H06	48	1	Bacteroidetes	Saprospiraceae	environmental	813
BDC2_A07	49	1	Bacteroidetes	Saprospiraceae	environmental	810
BDC2_B07	51	1	Proteobacteria	Alpha	environmental	819
BDC2_B08	52	1	Proteobacteria	Alpha	environmental	835
BDC2_C07	53	1	Acidobacteria	Acidobacteria-1	Acap group	811
BDC2_C08	54	1	Chloroflexi	Chloroflexi-1	Chloroflexi 1a	822
BDC2_D07	55	1	Proteobacteria	Beta	Burkholderiales	805
BDC2_E07	57	1	Chloroflexi	ARD environmental group		810
BDC2_E08	58	1	Acidobacteria	environmental		810
BDC2_F07	59	1	Proteobacteria	Delta	Desulfuromonadales	820
BDC2_F08	60	1	Proteobacteria	Beta	environmental	813
BDC2_G08	62	1	Chloroflexi	ARD environmental group		828

Table A-IV 5. Deer Creek Snowmelt: ARB Divisions, continued

ARB Name	RFLP type	Number of RFLPs	ARB division	ARB subdivision		Bases
BDC2_H07	63	1	Proteobacteria	environmental		809
BDC2_H08	64	1	Actinobacteria	Acidimicrobidae	acidimicrobium	821
BDC2_A10	66	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	799
BDC2_B09	67	1	Bacteroidetes	Saprospiraceae	environmental	829
BDC2_B10	68	1	Bacteroidetes	Saprospiraceae	environmental	808
BDC2_C09	69	1	Proteobacteria	Beta	Burkholderiales	816
BDC2_C10	70	1	bacterial environmental samples			799
BDC2_D09	71	1	Nitrospira	environmental		820
BDC2_D10	72	1	Proteobacteria	Alpha	Sphingomonadales	825
BDC2_E09	73	1	Proteobacteria	Delta	Desulfuromonadales	823
BDC2_E10	74	1	Acidobacteria	Acidobacteria-1	Acap group	806
BDC2_F09	75	1	Firmicutes	Chlostridium et al	caloramator et al	829
BDC2_F10	76	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	512
BDC2_G09	77	1	Chloroflexi	Chloroflexi-2	environmental	803
BDC2_G10	78	1	Chloroflexi	Chloroflexi-1	Chloroflexi 1a	809
BDC2_H09	79	1	OP11	environmental		826
BDC2_H10	80	1	Proteobacteria	Alpha	Acetobacteraceae	807
BDC2_A11	81	1	Proteobacteria	Delta	Desulfuromonadales	808
BDC2_A12	82	1	Proteobacteria	Alpha	Rhodobacterales	784
BDC2_B11	83	1	Firmicutes	Chlostridium et al	caloramator et al	598
BDC2_B12	84	1	Bacteroidetes	Saprospiraceae	environmental	821
BDC2_C11	85	1	Proteobacteria	Beta	environmental	828
BDC2_C12	86	1	Bacteroidetes	Flexibacteraceae	environmental	843
BDC2_D11	87	1	Proteobacteria	Beta	Methylophilus et al.	817
BDC2_D12	88	1	Actinobacteria	Rubrobacteridae	MC4	800
BDC2_E12	90	1	Euryarchaeaota	Thermoplasta	E2	814
BDC2_F11	91	1	Actinobacteria	Acidimicrobidae	acidimicrobium	805
BDC2_F12	92	1	Proteobacteria	Gamma	γ-proteobacteria A	800
BDC2_G11	93	1	Bacteroidetes	Saprospiraceae	environmental	832
BDC2_H11	95	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	819
BDC2_H12	96	1	Firmicutes	Butycivibrio		822

Table A-IV 6. Deer Creek Summer Long Band: Best BLAST Matches for Partials

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BDC3LE02	AF193514	36	92	Pseudomonas aeruginosa 16S rRNA partial
BDC3LG10	AF468431	870	97	Arctic sea ice bacterium ARK10270 16S rRNA partial
BDC3LH06	AJ243062	498	94	Gomphonema parvulum partial 18S rRNA gene
BDC3LA02	AJ519387	1031	94	Uncultured Holophaga sp. partial 16S rRNA, clone JG37-AG-112
BDC3LC01	AB016812	799	96	Hyphomicrobium methylovorum gene for 16S rRNA, partial

Table A-IV 7. Deer Creek Snowmelt: Best BLAST Matches (Accession numbers are not available)

ARB Name	RFLP type	Number of RFLPs	Bit Score	% ID	Best Blast Match
BDC2_A01	1	1	1564	98	Uncultured delta proteobacterium partial 16S rRNA clone
BDC2_A02	2	1	1084	92	Uncultured bacterium clone RCP2-2 16S rRNA partial
BDC2_B01	3	1	1610	99	Uncultured alpha proteobacterium partial 16S rRNA clone
BDC2_B02	4	1	1378	96	Uncultured delta proteobacterium partial 16S rRNA clone
BDC2_C01	5	1	1491	98	uncultured eubacterium, partial 16S rRNA clone WD2102
BDC2_C02	6	1	1279	94	uncultured eubacterium, partial 16S rRNA clone WD283
BDC2_D01	7	1	303	88	Uncultured beta proteobacterium clone Orbal D42 16S rRNA
BDC2_E01	9	1	1225	93	P.manganicum 16S rDNA
BDC2_E02	10	1	1306	94	Frankia sp. 16S ribosomal RNA (16S rRNA) gene
BDC2_F01	11	1	622	88	Selenomonas sp. oral clone DD020 16S rRNA partial
BDC2_F02	12	1	1271	94	Uncultured bacterium clone MB-A2-100 16S rRNA, partial
BDC2_G01	13	1	1417	95	Uncultured delta proteobacterium MERTZ_2CM_331 16S rRNA
BDC2_H01	15	1	1227	93	Agricultural soil bacterium clone SC-I-12, 16S rRNA gene
BDC2_H02	16	1	1352	96	Unidentified alpha proteobacterium OPT5 16S rRNA
BDC2_A04	18	1	1622	99	Bradyrhizobium genosp. P 16S rRNA gene
BDC2_B04	20	1	1507	98	Beta proteobacterium Wuba72 16S rRNA partial
BDC2_E04	26	1	1556	99	Uncultured Acidobacterium group clone partial 16S rRNA
BDC2_F03	27	1	1203	93	Uncultured bacterium clone GOUTB18 16S rRNA partial
BDC2_F04	28	1	1128	92	Uncultured bacterium clone HP1B19 16S rRNA partial
BDC2_G03	29	1	1285	96	Uncultured bacterium PHOS-HE21 16S rRNA partial
BDC2_G04	30	1	400	91	Microscilla sericea gene for 16S rRNA, strain:IFO 15983
BDC2_H03	31	1	1342	96	Actinomyces species 16S ribosomal RNA (isolate TM226)
BDC2_H04	32	1	1340	95	Thiobacillus plumbophilus 16S rRNA strain DSM 6690
BDC2_A05	33	1	1126	96	Proteobacterium sp. 16S rRNA clone 1200-34
BDC2_A06	34	1	1382	96	Uncultured bacterium clone FW145 16S rRNA partial
BDC2_B05	35	1	948	92	Leptothrix sp. 16S rRNA, partial
BDC2_D05	39	1	888	89	Pseudomonas stanieri DNA for 16S rRNA, strain ATCC 27130T
BDC2_C05	37	1	1031	91	beta proteobacterium TBW3, 16S rRNA gene
BDC2_E05	41	1	1209	93	Uncultured bacterium S28 16S rRNA partial
BDC2_F05	43	1	1629	99	Uncultured bacterium partial 16S rRNA clone JG34-KF-314
BDC2_F06	44	1	1326	95	Bacteroides cellulosolvens (ATCC 35603) 16S rRNA (16S rRNA)
BDC2_G05	45	1	1368	96	Uncultured sludge bacterium S16 16S rRNA partial
BDC2_G06	46	1	860	88	unidentified sulfate reducing bacterium DSB-DSa99-6 partial 16S
BDC2_H06	48	1	1489	98	P.manganicum 16S rDNA
BDC2_A07	49	1	1362	95	Sphingomonas sp. strain B28161 16S rRNA gene
BDC2_B07	51	1	1415	96	Uncultured γ-proteobacterium 16S rRNA clone BIsiii14
BDC2_B08	52	1	379	89	Uncultured soil bacterium clone C06 16S rRNA partial
BDC2_C07	53	1	1130	97	Uncultured beta proteobacterium clone NJ1 16S rRNA
BDC2_C08	54	1	1057	91	Uncultured bacterium clone RB7C6 16S rRNA partial
BDC2_D07	55	1	1507	99	Uncultured bacterium clone a13101 16S rRNA partial
BDC2_E07	57	1	1540	98	Uncultured bacterium partial 16S rRNA clone JG34-KF-135
BDC2_E08	58	1	186	91	Uncultured bacterium PENDANT-24 16S rRNA partial
BDC2_F07	59	1	1142	92	Uncultured bacterium FukuS59 16S rRNA gene
BDC2_F08	60	1	1497	98	Uncultured alpha proteobacterium partial 16S rRNA clone

Table A-IV 7. Deer Creek Snowmelt: Best BLAST Matches (Accession numbers are not available), continued

ARB Name	RFLP type	Number of RFLPs	Bit Score	% ID	Best Blast Match
BDC2_G08	62	1	1354	96	Uncultured bacterium clone FW145 16S rRNA partial
BDC2_H07	63	1	1289	93	Uncultured verrucomicrobium DEV114 partial 16S rRNA clone
BDC2_H08	64	1	1080	91	Uncultured delta proteobacterium MERTZ_2CM_70 16S rRNA
BDC2_A10	66	1	1574	99	Uncultured CFB group bacterium gene for 16S rRNA, partial
BDC2_B09	67	1	1550	98	Uncultured Green Bay ferromanganous micronodule MNB2 16S
BDC2_B10	68	1	1059	91	Uncultured archaeon WCHD3-30 16S rRNA partial
BDC2_C09	69	1	928	91	Bacterium Ellin325 16S rRNA, partial
BDC2_C10	70	1	1352	96	Uncultured bacterium PHOS-HE31 16S rRNA partial
BDC2_D09	71	1	837	91	Uncultured archaeon SW14 gene for 16S rRNA, partial
BDC2_D10	72	1	1239	94	Uncultured soil bacterium clone S165 16S rRNA partial
BDC2_E09	73	1	1316	95	Uncultured bacterium clone FW145 16S rRNA partial
BDC2_E10	74	1	930	89	uncultured bacterium SJA-68 16S rRNA clone SJA-68
BDC2_F09	75	1	1193	93	Uncultured bacterium clone FW48 16S rRNA partial
BDC2_F10	76	1	442	91	Uncultured bacterium clone RCP2-5 16S rRNA partial
BDC2_G09	77	1	289	94	Unidentified crenarchaeote clone pGrfB286 16S rRNA
BDC2_G10	78	1	1146	93	Uncultured bacterium clone FW2 16S rRNA partial
BDC2_H09	79	1	1612	99	Pseudomonas sp. PsC 16S rRNA, partial
BDC2_H10	80	1	1392	96	Uncultured bacterium clone HP1A30 16S rRNA partial
BDC2_A11	81	1	829	92	Uncultured soil bacterium PBS-25 partial 16S rRNA gene
BDC2_A12	82	1	1610	99	P.manganicum 16S rDNA
BDC2_B11	83	1	1116	92	Uncultured bacterium clone B27 16S rRNA, partial
BDC2_B12	84	1	730	88	Geobacter bremensis 16S rRNA, complete sequence
BDC2_C11	85	1	1360	96	Uncultured bacterium PHOS-HE31 16S rRNA partial
BDC2_C12	86	1	700	98	Uncultured Frankia sp. clone Lur44 16S rRNA partial
BDC2_D11	87	1	1025	93	Uncultured soil bacterium clone C0108 16S rNA gene, partial
BDC2_D12	88	1	1443	96	Uncultured bacterium PHOS-HE31 16S rRNA partial
BDC2_E12	90	1	1170	92	Agricultural soil bacterium clone SC-I-11, 16S rRNA gene
BDC2_F11	91	1	1312	95	Uncultured bacterium clone FW145 16S rRNA partial
BDC2_F12	92	1	1332	94	uncultured eubacterium, partial 16S rRNA clone WD283
BDC2_G11	93	1	1404	96	Alpha proteobacterium A0902 16S rRNA partial
BDC2_H11	95	1	1225	98	Haslea salstonica 16S rRNA, partial;
BDC2_H12	96	1	1017	93	Uncultured sludge bacterium A9 16S rRNA, partial

Table A-IV 8. Deer Creek Summer Short Band: ARB divisions

ARB Name	RFLP type	Number of RFLPs	ARB Division	ARB Subdivision		Number of Bases
BDC3SA01	1	1	Actinobacteria	Microbacteriaceae	environmental	805
BDC3SA02	2	1	Proteobacteria	Alpha	environmental	809
BDC3SB01	3	1	Proteobacteria	Alpha	Rhodobacterales	784
BDC3SB02	4	1	Actinobacteria	Acidimicrobidae	acidimicrobium	823
BDC3SC01	5	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	801
BDC3SC02	6	1	Proteobacteria	Alpha	environmental	769
BDC3SD01	7	1	Bacteroidetes	Flexibacteraceae	environmental	843
BDC3SD02	8	1	Proteobacteria	Beta	Burkholderiales	825
BDC3SE01	9	1	Proteobacteria	Alpha	environmental	837
BDC3SE02	10	1	Proteobacteria	Gamma	Envir. clone group	821
BDC3SF01	11	1	Actinobacteria	Intrasporangiaaceae	environmental	808
BDC3SF02	12	1	Bacteroidetes	Flexibacteraceae	environmental	759
BDC3SG01	13	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	795
BDC3SH01	15	1	Bacteroidetes	Flavobacteriales	Sporocytophaga	765
BDC3SH02	16	1	Actinobacteria	Acidimicrobidae	acidimicrobium	845
BDC3SA04	18	1	Acidobacteria	Acidobacteria-6	environmental	751
BDC3SB03	19	1	Acidobacteria	Acidobacteria-3	environmental	822
BDC3SB04	20	1	TM7	TM7-1		795
BDC3SE04	26	1	Proteobacteria	Alpha	environmental	816
BDC3SF03	27	1	Acidobacteria	Acidobacteria-6	environmental	756
BDC3SF04	28	1	Proteobacteria	Beta	Burkholderiales	809
BDC3SG03	29	1	Proteobacteria	Beta	environmental	814
BDC3SG04	30	1	Verrucomicrobia	VER-3	environmental	804
BDC3SH03	31	1	Firmicutes	Chlostridium et al	caloramator et al	573
BDC3SH04	32	1	Proteobacteria	Delta	Desulfuromonadales	802
BDC3SA05	33	1	Proteobacteria	Alpha	environmental	822
BDC3SA06	34	1	Proteobacteria	Gamma	g-proteobacteria A	785
BDC3SB05	35	1	Proteobacteria	Beta	Burkholderiales	817
BDC3SC05	37	1	Proteobacteria	Alpha	Rhodobacterales	803
BDC3SD05	39	1	Proteobacteria	Gamma	environmental	805
BDC3SE05	41	1	Proteobacteria	Alpha	environmental	810
BDC3SF05	43	1	Acidobacteria	Acidobacteria-6	environmental	798
BDC3SF06	44	1	Cyanobacteria	chloroplasts	chlorella chloroplast	832
BDC3SG05	45	1	Acidobacteria	Acidobacteria-6	environmental	814
BDC3SG06	46	1	Nitrospira	Ferromanganous		780
BDC3SH06	48	1	Bacteroidetes	Saprospiraceae	environmental	813
BDC3SA07	49	1	Bacteroidetes	Saprospiraceae	environmental	810
BDC3SB07	51	1	Proteobacteria	Alpha	environmental	819
BDC3SB08	52	1	Proteobacteria	Alpha	environmental	835
BDC3SC07	53	1	Acidobacteria	Acidobacteria-1	Acap group	811
BDC3SC08	54	1	Chloroflexi	Chloroflexi-1	Chloroflexi 1a	822
BDC3SD07	55	1	Proteobacteria	Beta	Burkholderiales	805
BDC3SE07	57	1	Chloroflexi	ARD envir. group		810
BDC3SE08	58	1	Acidobacteria	environmental		810

Table A-IV 8. Deer Creek Summer Short Band: ARB divisions, continued

ARB Name	RFLP type	Number of RFLPs	ARB Division	ARB Subdivision		Bases
BDC3SF07	59	1	Proteobacteria	Delta	Desulfuromonadales	820
BDC3SF08	60	1	Proteobacteria	Beta	environmental	813
BDC3SG08	62	1	Chloroflexi	ARD envir. group		828
BDC3SH07	63	1	Proteobacteria	environmental		809
BDC3SH08	64	1	Actinobacteria	Acidimicrobidae	acidimicrobium	821
BDC3SA10	66	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	799
BDC3SB09	67	1	Bacteroidetes	Saprospiraceae	environmental	829
BDC3SB10	68	1	Bacteroidetes	Saprospiraceae	environmental	808
BDC3SC09	69	1	Proteobacteria	Beta	Burkholderiales	816
BDC3SC10	70	1	bacterial environmental samples			799
BDC3SD09	71	1	Nitrospira	environmental		820
BDC3SD10	72	1	Proteobacteria	Alpha	Sphingomonadales	825
BDC3SE09	73	1	Proteobacteria	Delta	Desulfuromonadales	823
BDC3SE10	74	1	Acidobacteria	Acidobacteria-1	Acap group	806
BDC3SF09	75	1	Firmicutes	Chlostridium et al	caloramator et al	829
BDC3SF10	76	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	512
BDC3SG09	77	1	Chloroflexi	Chloroflexi-2	environmental	803
BDC3SG10	78	1	Chloroflexi	Chloroflexi-1	Chloroflexi 1a	809
BDC3SH09	79	1	OP11	environmental		826
BDC3SH10	80	1	Proteobacteria	Alpha	Acetobacteraceae	807
BDC3SA11	81	1	Proteobacteria	Delta	Desulfuromonadales	808
BDC3SB11	83	1	Firmicutes	Chlostridium et al	caloramator et al	598
BDC3SB12	84	1	Bacteroidetes	Saprospiraceae	environmental	821
BDC3SC12	86	1	Proteobacteria	Beta	environmental	828
BDC3SD11	87	1	Proteobacteria	Beta	Methylophilus et al.	817
BDC3SD12	88	1	Actinobacteria	Rubrobacteridae	MC4	800
BDC3SE12	90	1	Euryarchaeaota	Thermoplasta	E2	814
BDC3SF11	91	1	Actinobacteria	Acidimicrobidae	acidimicrobium	805
BDC3SF12	92	1	Proteobacteria	Gamma	g-proteobacteria A	800
BDC3SG11	93	1	Bacteroidetes	Saprospiraceae	environmental	832
BDC3SH11	95	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	819
BDC3SH12	96	1	Firmicutes	Butycivibrio		822

Table A-IV 9. Deer Creek Summer Short Band: Best BLAST Matches

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BDC3SA01	AF479342	1350	98	Glacial ice bacterium G200-C11 16S rRNA, partial
BDC3SA02	AJ224988	831	97	Unidentified eubacterium 16S rRNA clone GKS59
BDC3SB01	AJ247194	799	88	Asticcacaulis excentricus partial 16S rRNA gene for 16S ribosomal
BDC3SB02	AJ532727	1570	99	Uncultured bacterium partial 16S rRNA clone JG34-KF-316
BDC3SC01	AF418975	1396	97	Uncultured diatom clone HT2F1 16S rRNA, partial
BDC3SC02	AJ224988	765	96	Unidentified eubacterium 16S rRNA clone GKS59
BDC3SD01	AJ289964	1065	91	Bacterium isolate AH57 16S rRNA gene
BDC3SD02	AF351237	1483	98	Uncultured beta proteobacterium clone 8-11 16S rRNA
BDC3SE01	AJ534617	1489	98	Uncultured alpha proteobacterium partial 16S rRNA clone
BDC3SE02	AJ292676	1251	94	uncultured eubacterium partial 16S rRNA clone WD2124
BDC3SF01	AJ244674	1423	97	Janibacter like sp. V4.BO.43 partial 16S rRNA marine isolate
BDC3SF02	AF502211	1166	94	Uncultured bacterium clone HP1A92 16S rRNA partial
BDC3SG01	Z67753	1338	96	Odontella sinensis complete chloroplast genome
BDC3SH01	AF529117	1253	95	Uncultured Bacteroidetes bacterium clone FTLM155 16S rRNA
BDC3SH02	AF186411	1047	93	Uncultured sponge symbiont PAUC32f 16S rRNA partial
BDC3SA04	AF368180	1285	96	Uncultured Acidobacterium group clone SBR1013 16S rRNA
BDC3SB03	AF498724	957	89	Bacterium Ellin342 16S rRNA, partial
BDC3SB04	AF507687	993	90	Uncultured soil bacterium clone C129 16S rRNA partial
BDC3SE04	AJ532709	1580	99	Uncultured alpha proteobacterium partial 16S rRNA clone
BDC3SF03	AF523987	1156	95	Uncultured bacterium clone FW112 16S rRNA partial
BDC3SF04	AF407387	1465	97	Uncultured bacterium clone RB7C10 16S rRNA partial
BDC3SG03	AF293006	1199	94	Uncultured Green Bay ferromanganous micronodule MND1 16S
BDC3SG04	U62837	1092	94	Unidentified eubacterium RB14 16S rRNA, partial
BDC3SH03	X97432	1065	98	C.vincentii 16S rRNA gene
BDC3SH04	Y19190	1447	97	Geobacter sp. 16S rRNA strain CdA-2
BDC3SA05	AJ518761	1550	98	Uncultured alpha proteobacterium partial 16S rRNA clone
BDC3SA06	M95657	1059	92	Methylococcus luteus 16S ribosomal RNA
BDC3SB05	AY050600	1562	99	Uncultured bacterium clone GOUTB17 16S rRNA partial
BDC3SC05	AJ224988	1453	98	Unidentified eubacterium 16S rRNA clone GKS59
BDC3SD05	AF424161	1189	94	Uncultured gamma proteobacterium MERTZ_21CM_15 16S rRNA
BDC3SE05	AJ532705	1332	95	Uncultured alpha proteobacterium partial 16S rRNA clone
BDC3SF05	AF368180	1362	96	Uncultured Acidobacterium group clone SBR1013 16S ribosomal
BDC3SF06	AF278746	924	95	Koliella spiculiformis 16S small subunit rRNA
BDC3SG05	AY102321	1479	98	Uncultured bacterium clone a13114 16S rRNA, partial
BDC3SG06	AB096215	676	94	Gamma proteobacterium Y-134 gene for 16S rRNA, partial
BDC3SH06	AF314430	1384	96	Uncultured bacterium PHOS-HE31 16S rRNA, partial
BDC3SA07	AF314430	1441	97	Uncultured bacterium PHOS-HE31 16S rRNA, partial
BDC3SB07	AJ518761	1530	98	Uncultured alpha proteobacterium partial 16S rRNA clone
BDC3SB08	AF507705	726	93	Uncultured soil bacterium clone S165 16S rRNA partial
BDC3SC07	AF047646	1554	99	Uncultured eubacterium TRB82 16S rRNA partial
BDC3SC08	AF524014	1193	93	Uncultured bacterium clone FW48 16S rRNA, partial
BDC3SD07	AF414585	1501	98	Uncultured bacterium clone P3OU-26 16S rRNA partial
BDC3SE07	AF507681	460	90	Uncultured soil bacterium clone C0119 16S rRNA partial
BDC3SE08	AF424324	1197	93	Uncultured bacterium MERTZ_21CM_279 16S rRNA partial
BDC3SF07	AY013648	1213	97	Uncultured Banisveld landfill bacterium BVB33 16S rRNA

Table A-IV 9. Deer Creek Summer Short Band: Best BLAST Matches, continued

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BDC3SF08	AF293006	1400	97	Uncultured Green Bay ferromanganous micronodule MND1 16S
BDC3SG08	AF507681	460	90	Uncultured soil bacterium clone C0119 16S rRNA partial
BDC3SH07	AJ532715	1479	98	Uncultured delta proteobacterium partial 16S rRNA clone
BDC3SH08	AF523912	1003	91	Uncultured bacterium clone RCP1-37 16S rRNA partial
BDC3SA10	AF514854	1479	98	Haslea salstonica 16S rRNA partial;
BDC3SB09	AF314430	1429	97	Uncultured bacterium PHOS-HE31 16S rRNA, partial
BDC3SB10	AF314430	1421	97	Uncultured bacterium PHOS-HE31 16S rRNA, partial
BDC3SC09	AY050601	1469	97	Uncultured bacterium clone GOUTB18 16S rRNA partial
BDC3SC10	AF428801	381	89	Uncultured bacterium clone CR98-5-71 16S rRNA,
BDC3SD09	AF524008	1463	97	Uncultured bacterium clone FW114 16S rRNA partial
BDC3SD10	AF408323	1156	92	Uncultured Sphingomonas sp. clone KL-2-4-7 16S rRNA
BDC3SE09	Y19190	1505	98	Geobacter sp. 16S rRNA strain CdA-2
BDC3SE10	AF498692	1493	98	Bacterium Ellin310 16S rRNA, partial
BDC3SF09	AJ506120	1574	99	Clostridium bowmanii 16S rRNA type strain DSM 14206-16
BDC3SF10	Z67753	930	98	Odontella sinensis complete chloroplast genome
BDC3SG09	AF507694	1019	91	Uncultured soil bacterium clone S095 16S rRNA partial
BDC3SG10	AF234759	1366	96	Uncultured sludge bacterium S16 16S rRNA, partial
BDC3SH09	AF540834	450	94	Uncultured bacterium clone ME24-1 16S rRNA partial
BDC3SH10	AF253412	1328	96	Acidocella sp. WJB-3 16S rRNA, partial
BDC3SA11	Y19190	1475	98	Geobacter sp. 16S rRNA strain CdA-2
BDC3SB11	AJ506120	1067	97	Clostridium bowmanii 16S rRNA type strain DSM 14206-16
BDC3SB12	AY124340	1094	91	Bacterium CS57 16S rRNA, partial
BDC3SC12	AY133064	930	96	Uncultured beta proteobacterium clone ccs265 16S rRNA
BDC3SD11	AJ532688	1548	98	Uncultured beta proteobacterium partial 16S rRNA clone
BDC3SD12	AF234119	640	93	Uncultured bacterium Number0649-1G9 16S rRNA, partial
BDC3SE12	AF050616	1415	96	Uncultured archaeon WCHD3-02 16S rRNA partial
BDC3SF11	D87974	991	97	Nocardioides sp. DNA for 16S rRNA
BDC3SF12	AB015603	1285	95	Methylomonas sp. KSPIII gene for 16S rRNA, partial
BDC3SG11	AF050561	551	96	Uncultured eubacterium WCHD3-88 16S rRNA partial
BDC3SH11	Z67753	1409	96	Odontella sinensis complete chloroplast genome
BDC3SH12	AF427938	825	90	Uncultured bacterium clone 6A 16S rRNA, partial

Table A-IV 9. Deer Creek Summer Short Band: Best BLAST Matches for Partials

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BDC3SA09	AJ244674	1098	96	Janibacter like sp. V4.BO.43 partial 16S rRNA marine isolate
BDC3SC04	AF173823	527	97	Uncultured Antarctic bacterium LB3-81 16S rRNA,
BDC3SD03	AY121822	656	97	Aulacoseira skvortzowii 18S rRNA, partial
BDC3SD04	AJ422171	464	95	uncultured beta proteobacterium 16S rRNA clone Spb266
BDC3SD08	AL591983	38	100	Listeria monocytogenes strain EGD, complete genome segment 11/12
BDC3SE03	AF414588	827	99	Uncultured bacterium clone P3OB-42 16S rRNA partial
BDC3SG07	AJ534638	1421	97	Uncultured Nitrospirae bacterium partial 16S rRNA clone
BDC3SH05	AF523953	1033	97	Uncultured bacterium clone FW66 16S rRNA, partial
BDC3SA03	AJ519630	644	94	Uncultured delta proteobacterium partial 16S rRNA clone
BDC3SA08	AF499430	1245	98	Uncultured bacterium clone BL-57 16S rRNA, partial
BDC3SA12	AF507684	502	90	Uncultured soil bacterium clone S03 16S rRNA partial
BDC3SC03	AF407387	827	98	Uncultured bacterium clone RB7C10 16S rRNA partial
BDC3SC11	L26447	551	96	Homalozoon vermiculare ribosomal RNA small subunit
BDC3SE11	X64380	331	87	Bacterium (soil clone MC31) 16S rDNA
BDC3SG02	X68173	232	88	C.botulinum type B (Eklund 17B ATCC25765) rrn gene for 16S rRNA
BDC3SG12	AF314430	1203	94	Uncultured bacterium PHOS-HE31 16S rRNA partial

Table A-IV 10. Deer Creek Summer Long Band: ARB Divisions

ARB Name	RFLP type	Number of RFLPs	ARB Division	ARB Subdivision		Bases
BDC3LA01	1	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	792
BDC3LB01	3	1	Proteobacteria	Alpha	Bradyrhizobiaceae	793
BDC3LB02	4	1	OP5	environmental		770
BDC3LC02	6	1	Proteobacteria	Beta	Burkholderiales	758
BDC3LD01	7	1	Proteobacteria	Beta	environmental	827
BDC3LD02	8	1	Actinobacteria	Micrococcaceae	Arthrobacter	803
BDC3LE01	9	1	Proteobacteria	Beta	Burkholderiales	782
BDC3LF01	11	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	749
BDC3LF02	12	1	Proteobacteria	Beta	Burkholderiales	795
BDC3LG01	13	1	Bacteroidetes	Saprospiraceae	environmental	769
BDC3LG02	14	1	Acidobacteria	Acidobacteria-4	environmental	755
BDC3LH01	15	1	Acidobacteria	Acidobacteria-6	environmental	800
BDC3LH02	16	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	808
BDC3LA03	17	1	Proteobacteria	Delta	Desulfuromonadales	801
BDC3LA04	18	1	Acidobacteria	Acidobacteria-3	environmental	843
BDC3LB03	19	1	Proteobacteria	Beta	Burkholderiales	735
BDC3LB04	20	1	Eucaryotes	Heterokonta		971
BDC3LC03	21	1	Proteobacteria	Beta	environmental	789
BDC3LC04	22	1	Proteobacteria	Beta	Burkholderiales	760
BDC3LD03	23	1	Bacteroidetes	Saprospiraceae	environmental	759
BDC3LD04	24	1	Proteobacteria	Gamma	γ-proteobacteria A	746
BDC3LE03	25	1	Proteobacteria	environmental		831
BDC3LE04	26	1	Proteobacteria	Alpha	Rhodobacterales	745
BDC3LF03	27	1	Chlorobi	environmental		753
BDC3LF04	28	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	768
BDC3LG03	29	1	Proteobacteria	Alpha	environmental	767
BDC3LG04	30	1	Bacteroidetes	Saprospiraceae	environmental	747
BDC3LH03	31	1	Proteobacteria	Delta	Myxococcales	827
BDC3LH04	32	1	Proteobacteria	Beta	Burkholderiales	778
BDC3LA05	33	1	termite gut 1	environmental		832
BDC3LA06	34	1	bacterial environmental samples			818
BDC3LB05	35	1	Eucaryotes	environmental		970
BDC3LB06	36	1	Proteobacteria	Beta	Burkholderiales	785
BDC3LC05	37	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	814
BDC3LC06	38	1	Proteobacteria	Delta	Myxococcales	786
BDC3LD05	39	1	Proteobacteria	Beta	Burkholderiales	756
BDC3LD06	40	1	bacterial environmental samples			733
BDC3LE05	41	1	Bacteroidetes	Flavobacteriales	Sporocytophaga	775
BDC3LE06	42	1	Proteobacteria	Alpha	environmental	818
BDC3LF05	43	1	Verrucomicrobia	environmental		818
BDC3LF06	44	1	Proteobacteria	Beta	Burkholderiales	800
BDC3LG05	45	1	Eucaryotes	Heterokonta		960
BDC3LH05	47	1	Actinobacteria	Acidimicrobidae	acidimicrobium	796
BDC3LG06	46	1	Proteobacteria	Beta	Burkholderiales	840
BDC3LA07	49	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	814

Table A-IV 10. Deer Creek Summer Long Band: ARB Divisions

ARB Name	RFLP type	Number of RFLPs	ARB Division	ARB Subdivision		Bases
BDC3LA08	50	1	Verrucomicrobia	environmental		845
BDC3LB07	51	1	Proteobacteria	Beta	environmental	766
BDC3LB08	52	1	Acidobacteria	Acidobacteria-6	environmental	777
BDC3LC07	53	1	Proteobacteria	Beta	environmental	765
BDC3LC08	54	1	Proteobacteria	Alpha	Rhodobacterales	749
BDC3LD07	55	1	Eucaryotes	Heterokonta		948
BDC3LD08	56	1	Acidobacteria	Acidobacteria-3	environmental	798
BDC3LE07	57	1	Bacteroidetes	Saprospiraceae	environmental	800
BDC3LE08	58	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	768
BDC3LG07	61	1	Actinobacteria	Rubrobacteridae	MC4	753
BDC3LG08	62	1	Bacteroidetes	Bacteroidales	environmental	546
BDC3LH07	63	1	Proteobacteria	Beta	Methylophilus et al.	813
BDC3LH08	64	1	Proteobacteria	Beta	Burkholderiales	783
BDC3LA09	65	1	Eucaryotes	environmental		957
BDC3LA10	66	1	Eucaryotes	Apicomplexa		969
BDC3LB09	67	1	Proteobacteria	Alpha	environmental	844
BDC3LB10	68	1	Chlorobi	environmental		780
BDC3LC09	69	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	553
BDC3LC10	70	1	Actinobacteria	Acidimicrobidae	acidimicrobium	744
BDC3LD09	71	1	Proteobacteria	Alpha	Sphingomonadales	755
BDC3LD10	72	1	Proteobacteria	Alpha	environmental	744
BDC3LE09	73	1	Eucaryotes	environmental		939
BDC3LE10	74	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	821
BDC3LF09	75	1	Proteobacteria	Beta	Burkholderiales	773
BDC3LF10	76	1	Proteobacteria	Beta	Burkholderiales	771
BDC3LG09	77	1	Proteobacteria	Beta	environmental	787
BDC3LH09	79	1	Proteobacteria	TM6	environmental	795
BDC3LH10	80	1	Actinobacteria	Propionibacterineae	environmental	741
BDC3LA11	81	1	Bacteroidetes	Saprospiraceae	environmental	761
BDC3LA12	82	1	Proteobacteria	Beta	Burkholderiales	824
BDC3LB11	83	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	799
BDC3LB12	84	1	Proteobacteria	Alpha	Sphingomonadales	781
BDC3LC11	85	1	Actinobacteria	Rubrobacteridae	MC4	760
BDC3LC12	86	1	Verrucomicrobia	VER-4	environmental	779
BDC3LD11	87	1	Chloroflexi	Chloroflexi-1	environmental	777
BDC3LD12	88	1	Proteobacteria	Beta	environmental	809
BDC3LE11	89	1	Proteobacteria	Alpha	Rhodobacterales	742
BDC3LE12	90	1	bacterial environmental samples			770
BDC3LF11	91	1	Proteobacteria	Alpha	environmental	882
BDC3LF12	92	1	Proteobacteria	Alpha	environmental	800
BDC3LG11	93	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	771
BDC3LG12	94	1	Verrucomicrobia	environmental		821
BDC3LH11	95	1	Planctomycetes	Gemmata	environmental	748
BDC3LH12	96	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	818

Table A-IV 11. Deer Creek Summer Long Band: Best BLAST Matches

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BDC3LA01	AF514850	1501	98	Haslea nipkowii 16S rRNA partial; chloroplast
BDC3LB01	Z94805	1493	98	Bradyrhizobium genosp. P 16S rRNA gene
BDC3LB02	AF419685	896	90	Uncultured bacterium CS_B038 16S rRNA partial
BDC3LC02	AY050601	1402	98	Uncultured bacterium clone GOUTB18 16S rRNA, partial
BDC3LD01	AJ007650	1530	98	Uncultured proteobacterium 16S rRNA clone 1405-9
BDC3LD02	AJ512504	1528	99	Arthrobacter nitroguaiacolicus 16S rRNA DSM 4924
BDC3LE01	AB008503	1090	92	Ultramicrobacterium str. MY14 gene for 16S rRNA, partial
BDC3LF01	AF454328	1433	99	Uncultured plastid clone ML310M-8 16S rRNA, partial
BDC3LF02	AB074945	1520	99	Uncultured beta proteobacterium 16S rRNA, partial
BDC3LG01	AY187354	1084	96	Uncultured bacterium clone csbio160215 16S rRNA
BDC3LG02	Z95712	1247	96	Bacterial species 16S rRNA gene (clone 32-19)
BDC3LH01	Z95733	1459	98	Bacterial species 16S rRNA gene (clone mb1228)
BDC3LH02	Z67753	1409	96	Odontella sinensis complete chloroplast genome
BDC3LA03	AF019929	1471	98	Geobacter sp. Ala-6 16S rRNA partial
BDC3LA04	AF523980	1203	93	Uncultured bacterium clone FW14 16S rRNA partial
BDC3LB03	AF078763	1306	97	Acidovorax sp. IMI 357678 16S rRNA partial
BDC3LB04	AY180007	1550	96	Uncultured diatom clone CCW1 18S small subunit rRNA
BDC3LC03	AF407387	1120	92	Uncultured bacterium clone RB7C10 16S rRNA, partial
BDC3LC04	AF538929	1419	98	Variovorax sp. WDL1 16S rRNA partial
BDC3LD03	AF314430	1308	96	Uncultured bacterium PHOS-HE31 16S rRNA partial
BDC3LD04	AJ414655	1447	99	Methylobacter sp. SV96 16S ribosomal RNA, strain SV96
BDC3LE03	AJ534624	1439	97	Uncultured proteobacterium 16S rRNA JG36-TzT-56
BDC3LE04	AJ224988	1461	99	Unidentified eubacterium 16S rRNA clone GKS59
BDC3LF03	AF314435	1310	97	Uncultured bacterium PHOS-HE36 16S rRNA partial
BDC3LF04	Z67753	1346	96	Odontella sinensis complete chloroplast genome
BDC3LG03	AB089096	880	91	Uncultured alpha proteobacterium 16S rRNA, partial
BDC3LG04	AY187354	1221	96	Uncultured bacterium clone csbio160215 16S rRNA
BDC3LH03	AJ519631	1098	92	Uncultured delta proteobacterium 16S rRNA clone
BDC3LG07	AY121818	321	99	Aulacoseira subarctica 18S rRNA partial
BDC3LG08	AJ488070	1271	96	Uncultured bacterium partial 16S rRNA clone IA-16
BDC3LH04	X97070	1423	98	L.cholodnii 16S rRNA gene
BDC3LA05	AJ240996	1088	92	uncultured Holophaga/Acidobacterium Sva0777 16S rRNA
BDC3LA06	AJ519402	1435	96	Uncultured actinobacterium 16S rRNA, JG37-AG-121
BDC3LB05	AY180018	1338	94	Uncultured cercozoan clone CCW29 18S rRNA
BDC3LB06	AF468331	1409	97	Uncultured bacterium clone ARKMP-113 16S rRNA gene
BDC3LC05	AF514854	1505	98	Haslea salstonica 16S rRNA partial;
BDC3LC06	AJ519631	1176	94	Uncultured delta proteobacterium partial 16S rRNA, clone
BDC3LD05	AY050601	1404	98	Uncultured bacterium clone GOUTB18 16S rRNA, partial
BDC3LD06	AF402974	805	89	Uncultured proteobacterium clone bacteriap14 16S rRNA
BDC3LE05	AF145849	1457	98	Metal-contaminated soil clone K20-54 16S rRNA, partial
BDC3LE06	AJ292596	1392	96	uncultured eubacterium partial 16S rRNA, clone WD236
BDC3LF05	AJ401114	1281	95	Uncultured verrucomicrobium DEV059 16S rRNA clone
BDC3LF06	AF418942	1532	99	Uncultured bacterium clone HTA10 16S rRNA, partial
BDC3LG05	AY121822	1762	98	Aulacoseira skvortzowii 18S rRNA partial
BDC3LG06	AF150702	1475	97	Elbe River snow isolate Iso21 16S rRNA partial

Table A-IV 11. Deer Creek Summer Long Band: Best BLAST Matches, continued

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BDC3LH05	AF468240	1483	98	Uncultured bacterium clone ARKCH2Br2-66 16S rRNA
BDC3LA07	Z67753	1407	96	Odontella sinensis complete chloroplast genome
BDC3LA08	AY192332	733	96	Uncultured Verrucomicrobia clone W2a-2A 16S rRNA
BDC3LB07	Y09967	1354	97	denitrifying bacterium 72Chol 16S rRNA gene
BDC3LB08	AJ532720	1437	98	Uncultured bacterium partial 16S rRNA, clone JG34-KF-135
BDC3LC07	AF293006	1170	94	Uncultured Green Bay ferromanganous micronod MND1 16S
BDC3LC08	AF502216	1350	97	Uncultured bacterium clone HP1B39 16S rRNA partial
BDC3LD07	AY180007	1582	97	Uncultured diatom clone CCW1 18S rRNA
BDC3LD08	AJ519380	1225	97	Uncultured Holophaga sp. 16S rRNA clone JG37-AG-74
BDC3LE07	AJ252615	1332	96	Agricultural soil bacterium clone SC-I-12, 16S rRNA (partial)
BDC3LE08	AF514854	1407	98	Haslea salstonica 16S rRNA partial
BDC3LF07	AF407394	1136	92	Uncultured bacterium clone RB9C3 16S rRNA partial
BDC3LF08	AF402974	726	89	Uncultured proteobacterium clone bacteriap14 16S rRNA
BDC3LA09	AJ506034	1683	97	Uncultured rhizosphere cercozoan partial 18S rRNA clone
BDC3LA10	AB000912	795	92	Tridacna hemolymph apicomplexan gene for 18S rRNA
BDC3LB09	AF142936	186	91	Uncultured bacterium PENDANT-24 16S rRNA partial
BDC3LB10	AF314426	1396	97	Uncultured bacterium PHOS-HC15 16S rRNA partial
BDC3LC09	M82860	658	93	Olisthodiscus luteus chloroplast 16S rRNA(16S rRNA) gene
BDC3LH07	AJ532688	1524	98	Uncultured beta proteobacterium partial 16S rRNA clone
BDC3LH08	AJ295516	842	89	Uncultured rape rhizosphere wr0070 partial 16S rRNA
BDC3LC10	AF543499	942	91	Uncultured bacterium clone ASL8 16S rRNA partial
BDC3LD09	AB074191	1207	95	Sphingomonas sp. HL7 gene for 16S rRNA, partial
BDC3LD10	AJ518761	1411	98	Uncultured alpha proteobacterium partial 16S rRNA clone
BDC3LE09	AF408230	777	90	Coleochaete soluta 18S small subunit rRNA partial
BDC3LE10	AY168726	870	98	Uncultured diatom clone HC-42P 16S rRNA partial
BDC3LF09	AF351237	1455	98	Uncultured beta proteobacterium clone 8-11 16S rRNA gene
BDC3LF10	AJ421928.2	1358	97	Uncultured beta proteobacterium partial 16S rRNA Elb168
BDC3LG09	AJ421936.2	854	94	Uncultured beta proteobacterium partial 16S rRNA Elb236
BDC3LH09	U81641.2	833	90	Unidentified eubacterium clone vadinBA07 16S rRNA gene
BDC3LH10	AB097215	1469	100	Propionibacterium acnes 16S rRNA, complete sequence
BDC3LA11	AF314430	1344	97	Uncultured bacterium PHOS-HE31 16S rRNA partial
BDC3LA12	AF006507	1231	96	Unidentified beta proteobacterium ps.6 16S rRNA
BDC3LB11	AJ007656	1132	96	Uncultured proteobacterium 16S rRNA clone 1404-40
BDC3LB12	AB074640	1524	99	Uncultured alpha proteobacterium gene for 16S rRNA, partial
BDC3LC11	AF468431	793	96	Arctic sea ice bacterium ARK10270 16S rRNA partial
BDC3LC12	AF075271.2	1180	94	Alterococcus agarolyticus 16S rRNA complete sequence
BDC3LD11	AJ009460	1398	97	uncultured bacterium SJA-35 16S rRNA clone SJA-35
BDC3LD12	AF293006	1227	94	Uncultured Green Bay ferromanganous micronod MND1 16S
BDC3LE11	AJ224988	1304	97	Unidentified eubacterium 16S rRNA clone GKS59
BDC3LE12	AF428801	541	89	Uncultured bacterium clone CR98-5-71 16S rRNA
BDC3LF11	AF277479	139	90	Uncultured alpha proteobacterium SIC.42340 16S rRNA
BDC3LF12	AJ318111	1354	96	Uncultured alpha proteobacterium 16S rRNA clone BIci23
BDC3LG11	AF514855	1376	97	Haslea wawrikae 16S rRNA partial; chloroplast
BDC3LG12	AY192334	823	96	Uncultured Verrucomicrobia bacterium clone W3-1G 16S rRNA
BDC3LH11	AF239693	1025	92	Gemmata-like str. CJuql4 16S rRNA partial
BDC3LH12	AF514854	1542	98	Haslea salstonica 16S rRNA partial;

Table A-IV 12. Snake River Snowcover Short Band: ARB Divisions

ARB Name	RFLP type	Number of RFLPs	ARB Division	ARB Subdivision		Bases
BSR1SA01	1	1	Proteobacteria	Alpha	Bradyrhizobiaceae	826
BSR1SA02	2	5	Actinobacteria	Acidimicrobidae	acidimicrobium	794
BSR1SB01	3	10	Acidobacteria	environmental		794
BSR1SC01	4	1	Acidobacteria	ARD clones		828
BSR1SD01	5	8	Acidobacteria	ARD clones		881
BSR1SD02	6	18	Actinobacteria	Acidimicrobidae	acidimicrobium	874
BSR1SE01	7	6	Planctomycetes	Gemmata	environmental	560
BSR1SH01	8	12	Proteobacteria	Alpha	Bradyrhizobiaceae	882
BSR1SG03	9	9	Acidobacteria	Acidobacteria-1	environmental	697
BSR1SB04	11	6	Proteobacteria	Alpha	environmental	800
BSR1SH04	12	1	Proteobacteria	Beta	Burkholderiales	819
BSR1SA06	13	1	Planctomycetes	Planctomyces	environmental	872
BSR1SD06	14	1	Proteobacteria	Gamma	Environ. clone group	835
BSR1SE06	15	2	Acidobacteria	environmental		697
BSR1SF06	16	1	Acidobacteria	Acidobacteria-1	Acap group	844
BSR1SD08	17	2	Proteobacteria	Beta	Burkholderiales	857
BSR1SE07	18	1	Bacteroidetes	Bacteroidales	environmental	821
BSR1SH09	20	3	Chloroflexi	ARD clones		792

Table A-IV 13. Snake River Snowcover Long Band: ARB Divisions

ARB Name	RFLP type	Number of RFLPs	ARB Division	ARB Subdivision		Bases
BSR1LD02	1	46	Eucaryotes	Heterokonta		976
BSR1LA02	2	3	Actinobacteria	Acidimicrobidae	acidimicrobium	857
BSR1LB01	3	2	ARD1	environmental		792
BSR1LB02	4	4	Proteobacteria	Alpha	Bradyrhizobiaceae	803
BSR1LC01	5	2	Eucaryotes	Heterokonta		1027
BSR1LC02	6	1	Eucaryotes	Heterokonta		950
BSR1LC03	8	1	Chloroflexi	ARD environmental group		848
BSR1LD03	9	2	Eucaryotes	Apicomplexa		984
BSR1LF03	10	2	Crenarchaeota	C1	C1a	829
BSR1LA06	11	12	Eucaryotes	environmental		989
BSR1LC05	12	2	Chloroflexi	ARD environmental group		
BSR1LC06	13	1	Eucaryotes	environmental		969
BSR1LD08	15	2	Eucaryotes	Heterokonta		963
BSR1LC08	16	1	Actinobacteria	Propionibacterineae	environmental	864
BSR1LE07	17	1	Eucaryotes	environmental		1010
BSR1LF07	18	1	Eucaryotes	Heterokonta		996
BSR1LG07	20	1	Chloroflexi	ARD environmental group		821
BSR1LA09	21	2	Chloroflexi	ARD environmental group		792
BSR1LC10	22	1	Planctomycetes	Isosphaera	environmental	805
BSR1LF11	26	1	Acidobacteria	Acidobacteria-1	Acap group	826

Table A-IV 14. Snake River Snowcover Short Band: Best BLAST Matches

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BSR1SA01	AY039016	1586	99	Bradyrhizobium sp. ORS 3260 16S rRNA partial
BSR1SA02	AF523917	1483	98	Uncultured bacterium clone RCP2-103 16S rRNA partial
BSR1SB01	AJ519372	1231	98	Uncultured Holophaga sp. partial 16S rRNA, clone JG37-AG-47
BSR1SC01	AJ519372	1548	99	Uncultured Holophaga sp. partial 16S rRNA, clone JG37-AG-47
BSR1SD01	AJ534634	1312	96	Uncultured Acidobacterium group bacterium partial 16S rRNA
BSR1SD02	AJ519394	1562	98	Uncultured actinobacterium partial 16S rRNA, clone JG37-AG-8
BSR1SE01	AF391976	848	94	Uncultured thermal soil bacterium clone YNPFFP32 16S rRNA
BSR1SH01	Z94805	1673	99	Bradyrhizobium genosp. P 16S rRNA gene
BSR1SG03	AJ519378	1176	96	Uncultured Holophaga sp. partial 16S rRNA, clone JG37-AG-67
BSR1SB04	AJ458486	1267	95	Methylosinus sporium partial 16S rRNA strain 8
BSR1SH04	AY050601	1435	97	Uncultured bacterium clone GOUTB18 16S rRNA partial
BSR1SA06	X81950	1604	98	Planctomyces sp. partial 16S rRNA gene (Schlesner 642)
BSR1SD06	AJ292673	1445	97	uncultured eubacterium partial 16S rRNA clone WD260
BSR1SE06	AJ292578	1053	94	uncultured eubacterium partial 16S rRNA clone WD228
BSR1SF06	AJ292578	1407	96	uncultured eubacterium partial 16S rRNA clone WD228
BSR1SD08	AF035054	1562	98	Beta proteobacterium B8 16S rRNA partial
BSR1SE07	AF050543	1429	97	Uncultured eubacterium WCHB1-32 16S rRNA partial
BSR1SH09	AF465676	355	90	Uncultured bacterium DGGE band YNPRH-B18 16S rRNA

Table A-IV 15. Snake River Snowcover Long Band: Best BLAST Matches

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BSR1LD02	M87336	1598	95	Synura spinosa 16S-like rRNA, complete
BSR1LA02	AF523917	1584	98	Uncultured bacterium clone RCP2-103 16SrRNA partial
BSR1LB01	AJ519409	1306	96	Uncultured bacterium 16S rRNA, clone JG37-AG-138
BSR1LB02	Z94805	1538	99	Bradyrhizobium geno sp. P 16S rRNA gene
BSR1LC01	M87336	1628	95	Synura spinosa 16S-like rRNA, complete
BSR1LC02	M87336	1568	97	Synura spinosa 16S-like rRNA, complete
BSR1LC03	AF465668	394	91	Uncultured bacterium DGGE band YNPRH-B10 16S rRNA
BSR1LD03	AF513227	595	93	Cryptosporidium sp. 18S rRNA partial
BSR1LF03	AB050208	1620	99	Uncultured archaeon SAGMA-D for 16S rRNA, partial
BSR1LA06	AJ418791	1041	96	Assulina muscorum 18S rRNA gene
BSR1LC05	AF507681	329	91	Uncultured soil bacterium clone C0119 16S rRNA
BSR1LC06	AY082996	985	95	Uncultured eukaryote clone RT5iin3 18S rRNA gene
BSR1LD08	M87336	1580	95	Synura spinosa 16S-like rRNA, complete
BSR1LC08	AB042289	1461	96	Propionibacterium acnes 16S rRNA, isolate:Number7168
BSR1LE07	AY082996	985	95	Uncultured eukaryote clone RT5iin3 18S rRNA gene
BSR1LF07	M87336	1590	95	Synura spinosa 16S-like rRNA, complete
BSR1LG07	AF507681	440	90	Uncultured soil bacterium C0119 16S rRNA, partial
BSR1LA09	AF465676	333	90	Uncultured bacterium DGGE band YNPRH-B18 16S rRNA
BSR1LC10	AJ292682	1352	96	uncultured eubacterium partial 16S rRNA, clone WD287
BSR1LD10	AJ410455	36	100	Chlamydomonas rubrifilum partial 18S rRNA, st. SAG 3.85
BSR1LF11	AF498733	1354	96	Bacterium Ellin351 16S rRNA partial

Table A-IV 16. Snake River Snowmelt Short Band: ARB Divisions

ARB Name	RFLP type	Number of RFLPs	ARB Division	ARB Subdivision		Bases
BSR2SA01	1	2	Cyanobacteria	chloroplasts	Euglena Chloroplasts	632
BSR2SA02	2	10	Acidobacteria	Acidobacteria-1	Acap group	820
BSR2SB02	4	1	Crenarchaeota	C1	C1c	833
BSR2SC01	5	2	ARD1	environmental		831
BSR2SC02	6	2	Proteobacteria	Gamma	Environ. clone group	822
BSR2SH06	8	3	Proteobacteria	Beta	Burkholderiales	828
BSR2SG01	9	1	Proteobacteria	Gamma	Environ. clone group	788
BSR2SG02	10	3	Chloroflexi	ARD environmental group		784
BSR2SH01	11	1	Chloroflexi	ARD environmental group		799
BSR2SA04	12	18	Proteobacteria	Alpha	environmental	843
BSR2SB03	13	2	Chloroflexi	ARD environmental group		630
BSR2SD03	14	7	Chloroflexi	ARD environmental group		789
BSR2SD04	15	2	Acidobacteria	environmental		815
BSR2SH04	16	1	Acidobacteria	environmental		796
BSR2SA05	17	5	Acidobacteria	Acidobacteria-1	environmental	854
BSR2SA06	18	6	Proteobacteria	Gamma	Xanthomonadales	787
BSR2SB06	19	2	Crenarchaeota	C1	C1c	818
BSR2SA07	20	2	OP8	environmental		802
BSR2SG07	22	2	Acidobacteria	ARD environmental group		825
BSR2SB10	23	1	Bacteroidetes	Saprospiraceae	environmental	793
BSR2SC09	24	1	Acidobacteria	ARD environmental group		846
BSR2SC10	25	1	Proteobacteria	Alpha	Acetobacteraceae	803
BSR2SH10	26	1	Acidobacteria	ARD environmental group		826
BSR2SA11	27	2	Proteobacteria	Delta	environmental	578
BSR2SB12	28	1	Acidobacteria	Acidobacteria-1	environmental	812
BSR2SD11	29	1	Proteobacteria	Delta	Myxococcales	825
BSR2SG12	30	1	Proteobacteria	Delta	environmental	828

Table A-IV 17. Snake River Snowmelt Long Band: Best BLAST Matches for Partials

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BSR2LB02	AP005283	32	100	Corynebacterium glutamicum ATCC 13032 DNA, complete genome, section 10/10
BSR2LF08	AF500325	48	90	Bacillus sp. PG3 16S rRNA partial
BSR2LB11	AJ519409	1174	96	Uncultured bacterium partial 16S rRNA, JG37-AG-138

Table A-IV 18. Snake River Snowmelt Short Band: Best BLAST Matches for Partials

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BSR2SB01	AJ534634	936	97	Uncultured Acidobacterium group 16S rRNA
BSR2SE04	M58830	101	87	Lactobacillus sanfrancisco 16S ribosomal RNA
BSR2SA08	AL939114	34	100	Streptomyces coelicolor A3(2) genome; segment 11/29

Table A-IV 19. Snake River Snowmelt Short Band: Best BLAST Matches

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BSR2SA01	AF494537	1059	99	Mycobacterium sp. BPC5 16S rRNA, partial
BSR2SA02	AF498733	1356	95	Bacterium Ellin351 16S rRNA, partial
BSR2SB02	AB055988	789	97	Uncultured archaeon WSB-6 gene for 16S rRNA
BSR2SC01	AJ519409	1405	97	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BSR2SC02	AJ292673	1544	98	uncultured eubacterium partial 16S rRNA clone WD260
BSR2SH06	AF407387	1461	97	Uncultured bacterium clone RB7C10 16S rRNA, partial
BSR2SG01	AJ292673	1209	97	uncultured eubacterium partial 16S rRNA clone WD260
BSR2SG02	AF465668	331	90	Uncultured bacterium DGGE band YNPRH-B10 16S rRNA,
BSR2SH01	AF465676	379	91	Uncultured bacterium DGGE band YNPRH-B18 16S rRNA,
BSR2SA04	AF523950	1296	95	Uncultured bacterium clone FW93 16S rRNA, partial
BSR2SB03	AF507681	335	91	Uncultured soil bacterium clone C0119 16S rRNA, partial
BSR2SD03	AF465668	359	92	Uncultured bacterium DGGE band YNPRH-B10 16S rRNA,
BSR2SD04	AF200696	1112	95	Uncultured Acidobacterium UA1 16S rRNA, partial
BSR2SH04	AF200696	1308	95	Uncultured Acidobacterium UA1 16S rRNA, partial
BSR2SA05	AJ519365	1267	95	Uncultured Holophaga sp. partial 16S rRNA clone JG37-AG-9
BSR2SA06	AJ532711	1291	95	Uncultured gamma proteobacterium partial 16S rRNA clone
BSR2SB06	AJ535127	779	99	Uncultured crenarchaeote partial 16S rRNA clone JG36-GR-44
BSR2SA07	AF050554	1320	95	Uncultured eubacterium WFeA1-35 16S rRNA, partial
BSR2SG07	AJ534634	1471	98	Uncultured Acidobacterium group bacterium partial 16S rRNA
BSR2SB10	AJ318106	1257	95	Uncultured bacterium 16S rRNA clone BIai2
BSR2SC09	AJ292580	1485	97	uncultured eubacterium partial 16S rRNA clone WD244
BSR2SC10	AJ292598	1332	96	uncultured eubacterium partial 16S rRNA clone WD248
BSR2SH10	AJ295656	1505	98	Uncultured bacterium KF-JG30-18 partial 16S rRNA gene
BSR2SA11	AF523886	963	96	Uncultured bacterium clone RCP2-54 16S rRNA, partial
BSR2SB12	AF523980	1132	93	Uncultured bacterium clone FW14 16S rRNA, partial
BSR2SD11	AF424226	1320	95	Uncultured delta proteobacterium MERTZ_2CM_331 16S rRNA
BSR2SG12	AF523886	1471	97	Uncultured bacterium clone RCP2-54 16S rRNA, partial

Table A-IV 20. Snake River Summer Long Band: Best BLAST Matches for Partials

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BSR3LH12	AY050601	1140	96	Uncultured bacterium clone GOUTB18 16S rRNA, partial
BSR3LF4	Y11555	56	95	Uncultured bacterium DA079 16S rRNA gene
BSR3LF12	AE016767	769	97	Escherichia coli CFT073 section 13 of 18 of genome
BSR3LF11	AJ292606	680	98	uncultured eubacterium partial 16S rRNA clone WD295
BSR3LE12	AJ296553	1072	99	Uncultured bacterium GR-WP33-36 16S rRNA, GR-WP33-36
BSR3LG8	AF526913	63	100	Bacillus sp. 34hs1 16S ribosomal RNA (rrn) gene, partial

Table A-IV 21. Snake River Snowcover Short Band: Best BLAST Matches for Partials

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BSR1SD09	AF514855	404	91	Haslea wawrikae 16S rRNA partial; chloroplast
BSR1SF05	AJ534634	690	93	Uncultured Acidobacterium group bacterium 16S rRNA

Table A-IV 22. Snake River Snowmelt Long Band: ARB Divisions

ARB Name	RFLP type	Number of RFLPs	ARB Division	ARB Subdivision		Bases
BSR2LA02	1	1	Proteobacteria	Delta	environmental	835
BSR2LF07	2	3	Proteobacteria	Alpha	Acetobacteraceae	846
BSR2LA11	4	11	Proteobacteria	Beta	Burkholderiales	836
BSR2LA07	5	3	Chloroflexi	ARD clone		883
BSR2LF09	6	9	Proteobacteria	Alpha	environmental	828
BSR2LD02	7	2	Actinobacteria	Acidimicrobidae	acidimicrobium	798
BSR2LG09	8	11	Proteobacteria	Gamma	Environ. clone group	844
BSR2LE02	9	2	Actinobacteria	Acidimicrobidae	acidimicrobium	818
BSR2LG01	10	6	Acidobacteria	ARD clone		824
BSR2LF02	11	1	Proteobacteria	Gamma	Xanthomonadales	768
BSR2LG02	12	1	Acidobacteria	environmental		833
BSR2LH05	13	4	Chloroflexi	ARD clone		812
BSR2LD07	14	2	Acidobacteria	Acidobacteria-3	environmental	802
BSR2LC03	15	1	Proteobacteria	Alpha	environmental	846
BSR2LH07	16	2	Acidobacteria	environmental		817
BSR2LE04	17	1	Actinobacteria	Rubrobacteridae	MC4	825
BSR2LD09	18	2	Crenarchaeota	C1	C1c	390
BSR2LG07	19	2	Proteobacteria	Beta	Burkholderiales	836
BSR2LH04	20	1	Proteobacteria	Gamma	environmental	840
BSR2LA05	21	1	ARD1	environmental		855
BSR2LC07	22	4	Proteobacteria	Beta	Burkholderiales	799
BSR2LE05	23	2	ARD1	environmental		829
BSR2LE06	24	1	Planctomycetes	Gemmata	environmental	874
BSR2LG06	25	1	Chloroflexi	ARD clone		778
BSR2LB07	26	2	Acidobacteria	environmental		817
BSR2LD08	27	1	Chloroflexi	ARD clone		803
BSR2LE07	28	1	Chloroflexi	ARD clone		847
BSR2LA09	30	1	Proteobacteria	Gamma	Environ. clone group	766
BSR2LB09	31	1	Chloroflexi	ARD clone		821
BSR2LC09	32	1	Planctomycetes	Planctomyces	environmental	849
BSR2LC10	33	1	ARD1	environmental		832
BSR2LD10	34	2	Bacteroidetes	Saprospiraceae	environmental	817
BSR2LD12	34		Bacteroidetes	Saprospiraceae	environmental	
BSR2LE09	35	1	Proteobacteria	Alpha	environmental	876
BSR2LH09	36	1	Actinobacteria	Acidimicrobidae	acidimicrobium	830
BSR2LA12	37	1	Acidobacteria	Acidobacteria-3	environmental	825
BSR2LC11	39	2	Actinobacteria	Acidimicrobidae	acidimicrobium	832
BSR2LE11	40	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	613
BSR2LF12	41	1	Verrucomicrobia	environmental		873
BSR2LH11	42	1	Actinobacteria	Acidimicrobidae	acidimicrobium	829

Table A-IV 23. Snake River Snowmelt Long Band: Best BLAST Matches

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BSR2LA02	AF523886	1304	94	Uncultured bacterium clone RCP2-54 16S rRNA, partial
BSR2LF07	AF465654	1372	95	Uncultured alpha proteobacterium YNPRH71B 16S rRNA,
BSR2LA11	RFLP 19		97	
BSR2LA07	AJ518761	1536	98	Uncultured alpha proteobacterium partial 16S rRNA clone
BSR2LF09	AF376024	1067	94	Acidisphaera sp. NO-15 16S rRNA, partial
BSR2LD02	AF543499	1124	92	Uncultured bacterium clone ASL8 16S rRNA, partial
BSR2LG09	AJ534627	1229	94	Uncultured proteobacterium 16S rRNA clone JG36-TzT-192
BSR2LE02	AF523917	1554	99	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BSR2LG01	AJ534634	1467	97	Uncultured Acidobacterium group bacterium partial 16S rRNA
BSR2LF02	AJ532711	1287	96	Uncultured gamma proteobacterium partial 16S rRNA clone
BSR2LG02	AJ519667	1304	94	Uncultured Holophaga sp. partial 16S rRNA, clone GuBH2-AG-47
BSR2LH05	AF465668	353	90	Uncultured bacterium DGGE band YNPRH-B10 16S rRNA
BSR2LD07	AJ292571	1279	95	uncultured eubacterium partial 16S rRNA clone WD205
BSR2LC03	Y18946	1338	95	Methylosinus sporium 16S rRNA gene
BSR2LH07	AJ519368	1477	98	Uncultured Holophaga sp. partial 16S rRNA, clone JG37-AG-31
BSR2LE04	AJ440237	1354	95	Conexibacter woesei partial 16S rRNA, type strain DSM 14684T
BSR2LD09	AJ428025	696	99	Uncultured crenarchaeote partial 16S rRNA, clone FHMa2
BSR2LG07	AF407387	1473	97	Uncultured bacterium clone RB7C10 16S rRNA, partial
BSR2LH04	AY050601	938	97	Uncultured bacterium clone GOUTB18 16S rRNA, partial
BSR2LA05	AJ519409	1443	97	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BSR2LC07	AF035054	1505	98	Beta proteobacterium B8 16S rRNA, partial
BSR2LE05	AJ519409	1390	96	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BSR2LE06	AF239693	1332	94	Gemmata-like str. CJuql4 16S rRNA, partial
BSR2LG06	AF507681	327	91	Uncultured soil bacterium clone C0119 16S rRNA, partial
BSR2LB07	AF523916	882	98	Uncultured bacterium clone RCP2-2 16S rRNA, partial
BSR2LD08	AF507681	488	92	Uncultured soil bacterium clone C0119 16S rRNA, partial
BSR2LE07	AF465676	402	91	Uncultured bacterium DGGE band YNPRH-B18 16S rRNA
BSR2LA09	AF050532	902	90	Uncultured eubacterium WCHA1-65 16S rRNA, partial
BSR2LB09	AF465668.	438	93	Uncultured bacterium DGGE band YNPRH-B10 16S rRNA
BSR2LC09	AJ231184	1096	91	Planctomyces maris (strain DSM 8797T) 16S rRNA partial
BSR2LC10	AJ519409	1453	97	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BSR2LD10	AJ318106	636	94	Uncultured bacterium 16S rRNA clone BIai2
BSR2LE09	AF523945	1312	94	Uncultured bacterium clone FW96 16S rRNA, partial
BSR2LH09	AF543499	1398	96	Uncultured bacterium clone ASL8 16S rRNA, partial
BSR2LA12	AF529104	1312	95	Uncultured Acidobacterium group clone FTL227 16SrRNA
BSR2LC11	AF523917	1564	99	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BSR2LE11	AF364560	856	93	Uncultured Chlamydiales bacterium clone P-1 16S rRNA,
BSR2LF12	AJ401128	817	94	Uncultured verrucomicrobium DEV020 partial 16S rRNA clone
BSR2LH11	X92709	1146	92	Actinomyces species 16S ribosomal RNA (isolate TM232)

Table A-IV 37. Pennsylvania Mine Snowmelt: ARB Divisions

ARB Name	RFLP type	# of RFLPs	ARB Division	ARB Subdivision		Bases
BPM2_A01	1	1	Actinobacteria	Acidimicrobidae	acidimicrobium	819
BPM2_H01	2	2	Proteobacteria	Alpha	Acetobacteraceae	809
BPM2_B02	3	7	Proteobacteria	Gamma	Xanthomonadales	814
BPM2_C01	4	23	Proteobacteria	Beta	Burkholderiales	812
BPM2_C02	5	2	Cyanobacteria	chloroplasts	Euglena chloroplasts	800
BPM2_D01	6	1	Bacteroidetes	Flavobacteriales	Sporocytophaga	822
BPM2_E01	7	1	Acidobacteria	Acidobacteria-1	Acap group	782
BPM2_F01	8	6	Proteobacteria	Alpha	environmental	830
BPM2_G01	9	1	Actinobacteria	Rubrobacteridae	MC4	814
BPM2_H02	10	1	Acidobacteria	Acidobacteria-1	Acap group	810
BPM2_A03	11	1	Proteobacteria	Beta	Burkholderiales	807
BPM2_A04	12	1	Proteobacteria	Beta	Burkholderiales	812
BPM2_B03	13	2	Bacteroidetes	Flexibacteraceae	environmental	798
BPM2_C03	14	1	Proteobacteria	Alpha	environmental	778
BPM2_D03	15	2	Proteobacteria	Alpha	environmental	652
BPM2_E03	16	1	Chloroflexi	ARD clone		793
BPM2_F03	18	1	Proteobacteria	Delta	environmental	822
BPM2_H03	19	2	Firmicutes	alicyclobacillaceae		810
BPM2_A05	20	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	824
BPM2_A06	21	3	Actinobacteria	Micrococcaceae	Arthrobacter	832
BPM2_C06	22	1	Proteobacteria	Alpha	Acetobacteraceae	827
BPM2_D06	23	1	Proteobacteria	Alpha	Acetobacteraceae	834
BPM2_E06	24	2	Verrucomicrobia	VER-1	environmental	813
BPM2_F06	25	1	Acidobacteria	Acidobacteria-1	Acap group	808
BPM2_G05	26	2	Planctomycetes	Gemmata	environmental	815
BPM2_H05	27	1	Proteobacteria	TM6	environmental	812
BPM2_H06	28	1	Actinobacteria	Microbacteriaceae	environmental	826
BPM2_B08	29	1	Actinobacteria	Intrasporangiaaceae	environmental	807
BPM2_D07	30	2	ARD2	environmental		817
BPM2_F08	31	1	Acidobacteria	Acidobacteria-1	Acap group	832
BPM2_G08	32	1	Chloroflexi	ARD clone		793
BPM2_H07	33	2	Proteobacteria	Beta	Burkholderiales	825
BPM2_A09	35	1	Acidobacteria	Acidobacteria-3	environmental	808
BPM2_A10	36	1	Actinobacteria	Corynebacterineae	Gordoniaceae	822
BPM2_B10	37	2	Proteobacteria	Gamma	Xanthomonadales	842
BPM2_D10	38	1	ARD1	environmental		806
BPM2_H10	39	1	Proteobacteria	Alpha	environmental	869
BPM2_B11	40	1	OP11	environmental		821
BPM2_C11	41	1	Proteobacteria	Gamma	Xanthomonadales	835
BPM2_C12	42	1	Proteobacteria	Gamma	Xanthomonadales	564
BPM2_E12	43	1	Firmicutes	bacilli	Planococcaceae	847
BPM2_G11	44	1	Proteobacteria	Delta	environmental	807
BPM2_G12	45	1	Proteobacteria	Gamma	Xanthomonadales	812
BPM2_H12	46	1	Proteobacteria	Gamma	Xanthomonadales	839
BPM2_B07	47	1	Proteobacteria	Gamma	Xanthomonadales	849

Table A-IV 24. Snake River Summer Short Band: ARB Divisions

ARB Name	RFLP type	Number of RFLPs	ARB Division	ARB Subdivision		Bases
BSR3SA01	1	1	Actinobacteria	Intrasporangiaaceae	environmental	839
BSR3SA02	2		Proteobacteria	Alpha	environmental	
BSR3SB07	2		Proteobacteria	Alpha	environmental	
BSR3SB08	2		Proteobacteria	Alpha	environmental	
BSR3SC03	2		Proteobacteria	Alpha	environmental	
BSR3SE04	2		Proteobacteria	Alpha	environmental	
BSR3SE05	2		Proteobacteria	Alpha	environmental	
BSR3SG01	2	7	Proteobacteria	Alpha	environmental	846
BSR3SA11	3		Proteobacteria	Gamma	Environ. clone group	
BSR3SB01	3	7	Proteobacteria	Gamma	Environ. clone group	847
BSR3SC11	3		Proteobacteria	Gamma	Environ. clone group	
BSR3SD09	3		Proteobacteria	Gamma	Environ. clone group	
BSR3SD11	3		Proteobacteria	Gamma	Environ. clone group	
BSR3SE02	3		Proteobacteria	Gamma	Environ. clone group	
BSR3SF03	3		Proteobacteria	Gamma	Environ. clone group	
BSR3SA05	4		Cyanobacteria	chloroplasts	Euglena Chloroplasts	
BSR3SA08	4		Cyanobacteria	chloroplasts	Euglena Chloroplasts	
BSR3SA09	4		Cyanobacteria	chloroplasts	Euglena Chloroplasts	
BSR3SB02	4	12	Cyanobacteria	chloroplasts	Euglena Chloroplasts	830
BSR3SB06	4		Cyanobacteria	chloroplasts	Euglena Chloroplasts	
BSR3SC05	4		Cyanobacteria	chloroplasts	Euglena Chloroplasts	
BSR3SC06	4		Cyanobacteria	chloroplasts	Euglena Chloroplasts	
BSR3SC07	4		Cyanobacteria	chloroplasts	Euglena Chloroplasts	
BSR3SC08	4		Cyanobacteria	chloroplasts	Euglena Chloroplasts	
BSR3SD08	4		Cyanobacteria	chloroplasts	Euglena Chloroplasts	
BSR3SF06	4		Cyanobacteria	chloroplasts	Euglena Chloroplasts	
BSR3SG06	4		Cyanobacteria	chloroplasts	Euglena Chloroplasts	
BSR3SC01	5	2	Acidobacteria	Acidobacteria-1	Acap group	827
BSR3SG09	5		Acidobacteria	Acidobacteria-1	Acap group	
BSR3SA07	6		Acidobacteria	Acidobacteria-1	Acap group	
BSR3SB04	6		Acidobacteria	Acidobacteria-1	Acap group	
BSR3SC02	6	5	Acidobacteria	Acidobacteria-1	Acap group	753
BSR3SD06	6		Acidobacteria	Acidobacteria-1	Acap group	
BSR3SD07	6		Acidobacteria	Acidobacteria-1	Acap group	
BSR3SD01	7	1	Proteobacteria	Gamma	Environ. clone group	864
BSR3SH01	8	2	Proteobacteria	Delta	Myxococcales	860
BSR3SD02	8		Proteobacteria	Delta	Myxococcales	
BSR3SE01	9	2	Actinobacteria	Acidimicrobidae	acidimicrobium	816
BSR3SE08	9		Actinobacteria	Acidimicrobidae	acidimicrobium	
BSR3SB09	11		Acidobacteria	ARD environmental group		
BSR3SF02	11	4	Acidobacteria	ARD environmental group		848
BSR3SF11	11		Acidobacteria	ARD environmental group		
BSR3SG03	11		Acidobacteria	ARD environmental group		
BSR3SH02	13	1	Proteobacteria	Beta	Burkholderiales	848

Table A-IV 24. Snake River Summer Short Band: ARB Divisions, continued

ARB Name	RFLP type	Number of RFLPs	ARB Division	ARB Subdivision		Bases
BSR3SG04	17	2	Acidobacteria	Acidobacteria-3	environmental	828
BSR3SD03	18	2	Acidobacteria	Acidobacteria-1	Acap group	839
BSR3SD04	19	1	Acidobacteria	Acidobacteria-1	Acap group	617
BSR3SE03	20	1	Proteobacteria	Alpha	environmental	804
BSR3SF04	21	1	ARD2	environmental		859
BSR3SH03	22	1	Acidobacteria	Acidobacteria-3	environmental	848
BSR3SH04	23	2	OP10	environmental		909
BSR3SB05	25	1	Acidobacteria	Acidobacteria-1	Acap group	838
BSR3SD05	26	1	Chloroflexi	ARD environmental group		799
BSR3SE07	27	2	Proteobacteria	Beta	environmental	790
BSR3SF05	28	1	Acidobacteria	environmental		811
BSR3SG05	29	1	Proteobacteria	Alpha	Acetobacteraceae	585
BSR3SH05	30	1	ARD1	environmental		852
BSR3SF07	31	1	Crenarchaeota	C4	C1c	868
BSR3SF08	32	1	Acidobacteria	Acidobacteria-3	environmental	853
BSR3SG08	33	1	Acidobacteria	Acidobacteria-3	environmental	836
BSR3SD12	34	2	Acidobacteria	Acidobacteria-1	Acap group	805
BSR3SA12	38	1	Actinobacteria	Acidimicrobidae	acidimicrobium	802
BSR3SB12	39	1	Chloroflexi	ARD environmental group		802
BSR3SC12	40	1	Proteobacteria	Beta	Burkholderiales	809
BSR3SE11	41	1	Acidobacteria	ARD environmental group		834
BSR3SE12	42	1	Bacteroidetes	Sphingobacteriaceae	environmental	846
BSR3SF12	43	1	Proteobacteria	Alpha	Acetobacteraceae	836
BSR3SG11	44	1	Actinobacteria	Microbacteriaceae	environmental	823
BSR3SH12	46	1	Actinobacteria	Acidimicrobidae	acidimicrobium	827

Table A-IV 25. Snake River Summer Short Band: Best BLAST Matches for Partials

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BSR3SF01	AJ292578	127	92	uncultured eubacterium partial 16S rRNA clone WD228
BSR3SG02	AJ401128	894	93	Uncultured verrucomicrobium DEV020 partial 16S rRNA clone
BSR3SA03	AF200699	1427	99	Uncultured Acidobacterium UA3 16S rRNA partial
BSR3SH11	AF371929	121	95	Uncultured bacterium clone p-4365-4Wa2 16S rRNA
BSR3SB03	AF523892	874	95	Uncultured bacterium clone RCP1-43 16S rRNA partial
BSR3SG12	AJ535125	52	89	Uncultured crenarchaeote partial 16S rRNA, clone JG36-GR-12
BSR3SE09	AP005280	38	100	Corynebacterium glutamicum ATCC 13032 DNA, section 7/10

Table A-IV 26. Snake River Snowcover Long Band: Best BLAST Matches for Partials

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BSR1LH02	AF100312	1055	94	Paramecium duboscqui 18S rRNA partial
BSR1LB11	AB016019	1235	98	Monoblepharis sp. 18S rRNA strain UBC 70-1, partial
BSR1LF09	AJ458468	664	93	Methylosinus sporium partial 16S rRNA strain 44/2
BSR1LH05	AF507869	58	96	Uncultured Bacteroidetes bacterium clone ML635J-15 16S rRNA

Table A-IV 27. Snake River Summer Short Band: Best BLAST Matches

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BSR3SA01	AJ244674	1463	97	Janibacter like sp. V4.BO.43 partial 16S rRNA marine isolate
BSR3SG01	Y18946	1332	95	Methylosinus sporium 16S rRNA gene
BSR3SB01	AJ292673	1461	97	uncultured eubacterium partial 16S rRNA clone WD260
BSR3SB02	AF418976	1178	92	Uncultured diatom clone HTAA8 16S rRNA, partial
BSR3SC01	AF351237	1437	97	Uncultured beta proteobacterium clone 8-11 16S rRNA,
BSR3SC02	AF498733	1239	95	Bacterium Ellin351 16S rRNA, partial
BSR3SD01	AJ534627	1130	93	Uncultured proteobacterium partial 16S rRNA, JG36-TzT-192
BSR3SH01	AF482687	1180	92	Myxobacterium KC 16S rRNA, partial
BSR3SE01	AF523917	1481	98	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BSR3SF02	AJ534634	1515	98	Uncultured Acidobacterium group partial 16S rRNA
BSR3SH02	AY050601	1507	97	Uncultured bacterium clone GOUTB18 16S rRNA partial
BSR3SA04	AJ292598	1076	94	uncultured eubacterium partial 16S rRNA clone WD248
BSR3SG04	AF529104	1328	95	Uncultured Acidobacterium group clone FTL227 16S rRNA
BSR3SD03	AJ292579	1457	97	uncultured eubacterium partial 16S rRNA clone WD243
BSR3SD04	AJ292578	874	94	uncultured eubacterium partial 16S rRNA clone WD228
BSR3SE03	AF523945	1253	94	Uncultured bacterium clone FW96 16S rRNA, partial
BSR3SF04	AJ292684	775	95	uncultured eubacterium partial 16S rRNA clone WD272
BSR3SH03	AJ519380	1439	96	Uncultured Holophaga sp. partial 16S rRNA, clone JG37-AG-74
BSR3SH04	AJ534634	1497	98	Uncultured Acidobacterium group partial 16S rRNA
BSR3SB05	AJ292578	1469	97	uncultured eubacterium partial 16S rRNA clone WD228
BSR3SD05	AF465676	383	91	Uncultured bacterium DGGE band YNPRH-B18 16S rRNA gene
BSR3SE07	AJ458486	789	95	Methylosinus sporium partial 16S rRNA strain 8
BSR3SF05	U41563	1536	99	Geothrix fermentans 16S rRNA partial
BSR3SG05	AF376021	1102	98	Acidocella sp. NO-12 16S rRNA, partial
BSR3SH05	AJ519409	1437	97	Uncultured bacterium partial 16S rRNA, clone JG37-AG-138
BSR3SF07	AF458635	932	98	Uncultured crenarchaeote 03_03a 16S rRNA, partial
BSR3SF08	AF523886	924	96	Uncultured bacterium clone RCP2-54 16S rRNA, partial
BSR3SG08	AJ519380	1398	96	Uncultured Holophaga sp. partial 16S rRNA, JG37-AG-74
BSR3SD12	AJ292578	1413	97	uncultured eubacterium partial 16S rRNA clone WD228
BSR3SA12	X92703	1520	98	Actinomyces species 16S ribosomal RNA (isolate TM208)
BSR3SB12	AF507680	557	85	Uncultured soil bacterium clone C022 16S rRNA, partial
BSR3SC12	AF289156	1487	98	Uncultured beta proteobacterium clone PRD01a008B 16S rRNA
BSR3SE11	AJ519372	1592	99	Uncultured Holophaga sp. partial 16S rRNA, JG37-AG-47
BSR3SE12	AJ295473	1435	96	Uncultured rape rhizosphere wr0012 partial 16S rRNA gene
BSR3SF12	AF465654	1296	95	Uncultured alpha proteobacterium YNPRH71B 16S rRNA
BSR3SG11	AF050573	1562	99	Uncultured eubacterium WCHB1-08 16S rRNA, partial
BSR3SH12	X92701	1524	98	Actinomyces species 16S ribosomal RNA (isolate TM177)

Table A-IV 28. Snake River Summer Long Band: ARB Divisions

ARB Name	RFLP type	Number of RFLPs	ARB Division	ARB Subdivision		Bases
BSR3LC4	1	4	Proteobacteria	Gamma	Environ. clone group	824
BSR3LA02	2	7	Proteobacteria	Beta	Burkholderiales	856
BSR3LB11	3	6	Proteobacteria	Beta	environmental	834
BSR3LB02	4	1	Acidobacteria	ARD environmental group		816
BSR3LH8	6	6	Proteobacteria	Alpha	Rhodobacterales	817
BSR3LF8	7	5	Proteobacteria	Alpha	environmental	858
BSR3LE6	8	3	ARD1	environmental		818
BSR3LC10	9	4	Acidobacteria	ARD environmental group		853
BSR3LG2	10	1	Acidobacteria	environmental		777
BSR3LG11	12	2	Proteobacteria	Delta	environmental	921
BSR3LA04	13	1	Acidobacteria	Acidobacteria-1	Acap group	844
BSR3LB03	14	1	Chloroflexi	ARD environmental group		837
BSR3LB04	15	1	Planctomycetes	Planctomyces	environmental	845
BSR3LE3	17	1	Acidobacteria	environmental		855
BSR3LE4	18	1	Proteobacteria	Alpha	environmental	842
BSR3LF6	21	2	Actinobacteria	Acidimicrobidae	acidimicrobium	800
BSR3LA06	24	1	Proteobacteria	Gamma	Environ. clone group	672
BSR3LB06	25	1	Acidobacteria	Acidobacteria-1	environmental	828
BSR3LC5	26	1	Chloroflexi	ARD environmental group		805
BSR3LF5	27	1	OP11	OP11-5	environmental	860
BSR3LG5	28	1	Acidobacteria	Acidobacteria-1	environmental	870
BSR3LG12	29	2	ARD1	environmental		909
BSR3LA08	30	1	Bacteroidetes	Flexibacteraceae	environmental	797
BSR3LB08	31	1	Proteobacteria	Gamma	Xanthomonadales	837
BSR3LD08	32	3	Proteobacteria	Alpha	Acetobacteraceae	845
BSR3LC8	33	1	Acidobacteria	Acidobacteria-1	environmental	843
BSR3LC11	34	3	Cyanobacteria	chloroplasts	Euglena Chloroplasts	792
BSR3LE8	35	1	Proteobacteria	Gamma	Environ. clone group	866
BSR3LD09	37	1	Chloroflexi	ARD environmental group		785
BSR3LD10	38	1	Acidobacteria	environmental		823
BSR3LE9	39	2	Proteobacteria	Gamma	Environ. clone group	
BSR3LF9	40	2	Chloroflexi	ARD environmental group		837
BSR3LG9	41	1	Actinobacteria	Acidimicrobidae	Acidimicrobium	854
BSR3LH9	42	1	Acidobacteria	Acidobacteria-1	Acap group	793
BSR3LA11	43	1	Bacteroidetes	Saprospiraceae	environmental	803
BSR3LA12	44	1	Acidobacteria	ARD environmental group		855
BSR3LB12	45	1	Acidobacteria	Acidobacteria-3	environmental	845
BSR3LC12	46	1	Bacteroidetes	Saprospiraceae	environmental	817
BSR3LD11	47	1	Cyanobacteria	chloroplasts	Euglena Chloropasts	794
BSR3LD12	48	1	Proteobacteria	Beta	environmental	836
BSR3LE11	49	1	Actinobacteria	Acidimicrobidae	acidimicrobium	840
BSR3LH11	51	1	Crenarchaeota	C1	C1a	827

Table A-IV 29. Snake River Summer Long Band: Best BLAST Matches

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BSR3LC4	AJ534627	1211	94	Uncultured proteobacterium partial 16S rRNA, clone JG36-TzT-192
BSR3LA02	AF407387	1491	97	Uncultured bacterium clone RB7C10 16S rRNA, partial
BSR3LB11	AF407387	1191	93	Uncultured bacterium clone RB7C10 16S rRNA, partial
BSR3LB02	AJ295657	1207	93	Uncultured bacterium KF/GS-JG36-31 partial 16S rRNA gene
BSR3LH8	AJ518780	844	91	Uncultured alpha proteobacterium partial 16S rRNA clone
BSR3LF8	AY039017	1239	96	Bradyrhizobium sp. ORS 3262 16S rRNA, partial
BSR3LE6	AJ519409	1360	96	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BSR3LC10	AJ519372	1606	99	Uncultured Holophaga sp. partial 16S rRNA clone JG37-AG-47
BSR3LG2	AF523892	1396	98	Uncultured bacterium clone RCP1-43 16S rRNA, partial
BSR3LG11	AF523902	1513	99	Uncultured bacterium clone RCP2-13 16S rRNA, partial
BSR3LA04	AJ292578	1542	98	uncultured eubacterium partial 16S rRNA clone WD228
BSR3LB03	AF465668	398	91	Uncultured bacterium DGGE band YNPRH-B10 16S rRNA,
BSR3LB04	AB015527	1552	98	Planctomyces sp. 16S rRNA strain BD1-23, partial
BSR3LD04	AJ244674	973	96	Janibacter like sp. V4.BO.43 partial 16S rRNA marine isolate
BSR3LE3	AF523892	1481	97	Uncultured bacterium clone RCP1-43 16S rRNA, partial
BSR3LE4	Y07582	1457	97	Uncultured bacterium DA067 16S rRNA gene
BSR3LF6	AJ292673	898	97	uncultured eubacterium partial 16S rRNA clone WD260
BSR3LA06	AJ292673	1178	97	uncultured eubacterium partial 16S rRNA clone WD260
BSR3LB06	AF200699	1534	98	Uncultured Acidobacterium UA3 16S rRNA, partial
BSR3LC5	AF465668	426	92	Uncultured bacterium DGGE band YNPRH-B10 16S rRNA,
BSR3LF5	AF540828	513	95	Uncultured bacterium clone DP11u4 16S rRNA, partial
BSR3LG5	AF498692	1380	95	Bacterium Ellin310 16S rRNA, partial
BSR3LG12	AJ519409	1437	97	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BSR3LA08	AF314419	1102	92	Uncultured bacterium PHOS-HE21 16S rRNA, partial
BSR3LB08	AB074730	1526	98	Gamma proteobacterium S-N(1)-6B gene for 16S rRNA, partial
BSR3LD08	AF376021	1600	99	Acidocella sp. NO-12 16S rRNA, partial
BSR3LC8	AJ519378	1620	99	Uncultured Holophaga sp. partial 16S rRNA clone JG37-AG-67
BSR3LC11	AF418975	1370	97	Uncultured diatom clone HT2F1 16S rRNA, partial
BSR3LE8	AJ292673	1584	98	uncultured eubacterium partial 16S rRNA clone WD260
BSR3LD09	AF507682	381	86	Uncultured soil bacterium clone C011 16S rRNA,
BSR3LD10	AF523892	783	97	Uncultured bacterium clone RCP1-43 16S rRNA, partial
BSR3LF9	AY100627	628	91	Uncultured bacterium clone cvf116080 16S rRNA,
BSR3LG9	AJ292673	940	98	uncultured eubacterium partial 16S rRNA clone WD260
BSR3LH9	AF498733	1174	94	Bacterium Ellin351 16S rRNA, partial
BSR3LA11	AJ318106	1279	95	Uncultured bacterium 16S rRNA clone BIai2
BSR3LA12	AJ534634	1477	97	Uncultured Acidobacterium group bacterium partial 16S rRNA
BSR3LB12	AY118153	1055	91	Uncultured Acidobacteria/Holophaga group clone Cart-N4 16S
BSR3LC12	AJ318106	1296	95	Uncultured bacterium 16S rRNA clone BIai2
BSR3LD11	AF523902	841	99	Uncultured bacterium clone RCP2-13 16S rRNA, partial
BSR3LD12	AY133069	1536	98	Uncultured beta proteobacterium clone ccs214 16S rRNA,
BSR3LE11	X92709	1166	92	Actinomyces species 16S ribosomal RNA (isolate TM232)
BSR3LH11	AB050208	1608	99	Uncultured archaeon SAGMA-D gene for 16S rRNA, partial

Table A-IV 30. Pennsylvania Mine Snowcover Short Band: ARB Divisions

ARB Name	RFLP type	Number of RFLPs	ARB division	ARB subdivision		Bases
BPM1SA01	1	1	Firmicutes	bacilli	staphylococcaceae	766
BPM1SA03	3	1	Actinobacteria	Propionibacterineae	environmental	826
BPM1SA04	4	1	Proteobacteria	Alpha	Acetobacteraceae	807
BPM1SA06	6	1	Proteobacteria	Beta	Burkholderiales	764
BPM1SA07	7	1	Acidobacteria	Acidobacteria-1	Acap group	826
BPM1SA08	8	1	Actinobacteria	Acidimicrobidae	acidimicrobium	798
BPM1SA09	9	1	Actinobacteria	Acidimicrobidae	acidimicrobium	773
BPM1SA10	10	1	Proteobacteria	Gamma	Xanthomonadales	780
BPM1SA11	11	1	Proteobacteria	Alpha	Acetobacteraceae	829
BPM1SB01	13	1	Proteobacteria	Gamma	γ-proteobacteria A	817
BPM1SB03	15	1	Proteobacteria	Gamma	Xanthomonadales	837
BPM1SB04	16	1	Actinobacteria	Acidimicrobidae	acidimicrobium	794
BPM1SB06	18	1	Proteobacteria	Gamma	Xanthomonadales	802
BPM1SB07	19	1	Proteobacteria	Alpha	Acetobacteraceae	826
BPM1SB08	20	1	Proteobacteria	Gamma	Xanthomonadales	808
BPM1SB09	21	1	Proteobacteria	Alpha	Acetobacteraceae	838
BPM1SB11	23	1	Acidobacteria	Acidobacteria-1	Acap group	783
BPM1SB12	24	1	Proteobacteria	Beta	Burkholderiales	810
BPM1SC01	25	1	Actinobacteria	Acidimicrobidae	acidimicrobium	816
BPM1SC05	29	1	Actinobacteria	Acidimicrobidae	acidimicrobium	843
BPM1SC06	30	1	Actinobacteria	Propionibacterineae	environmental	825
BPM1SC11	35	1	Proteobacteria	Gamma	Xanthomonadales	803
BPM1SD01	37	1	Proteobacteria	Beta	Burkholderiales	793
BPM1SD05	41	1	Proteobacteria	Beta	environmental	826
BPM1SD06	42	1	Proteobacteria	Gamma	Xanthomonadales	815
BPM1SD07	43	1	Actinobacteria	Acidimicrobidae	acidimicrobium	821
BPM1SD12	48	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	802
BPM1SE05	53	1	Proteobacteria	Gamma	Xanthomonadales	858
BPM1SE06	54	1	Proteobacteria	Gamma	Xanthomonadales	827
BPM1SE11	59	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	783
BPM1SE12	60	1	Proteobacteria	Beta	Burkholderiales	802
BPM1SF01	61	1	Proteobacteria	Gamma	Xanthomonadales	828
BPM1SF03	63	1	Acidobacteria	Acidobacteria-1	Acap group	795
BPM1SF06	66	1	Proteobacteria	Gamma	Xanthomonadales	826
BPM1SF07	67	1	ARD2	environmental		815
BPM1SF10	70	1	Verrucomicrobia	VER-3	environmental	830
BPM1SF11	71	1	Actinobacteria	Acidimicrobidae	acidimicrobium	823
BPM1SF12	72	1	Proteobacteria	Alpha	Acetobacteraceae	826
BPM1SG01	73	1	Proteobacteria	Alpha	Acetobacteraceae	821
BPM1SG02	74	1	Planctomycetes	Isosphaera	environmental	827
BPM1SG04	76	1	Proteobacteria	Gamma	Xanthomonadales	843
BPM1SG05	77	1	Actinobacteria	Acidimicrobidae	acidimicrobium	846
BPM1SG06	78	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	640
BPM1SG07	79	1	Actinobacteria	Propionibacterineae	environmental	824

Table A-IV 30. Pennsylvania Mine Snowcover Short Band: ARB Divisions

ARB Name	RFLP type	Number of RFLPs	ARB division	ARB subdivision		Bases
BPM1SG08	80	1	Proteobacteria	Gamma	Xanthomonadales	828
BPM1SG09	81	1	Actinobacteria	Propionibacterineae	environmental	827
BPM1SG10	82	1	Actinobacteria	Acidimicrobidae	acidimicrobium	802
BPM1SG12	84	1	Proteobacteria	Gamma	γ-proteobacteria A	821
BPM1SH03	87	1	Proteobacteria	Gamma	Xanthomonadales	835
BPM1SH04	88	1	Verrucomicrobia	VER-3	environmental	804
BPM1SH05	89	1	Proteobacteria	Alpha	Acetobacteraceae	817
BPM1SH06	90	1	Actinobacteria	Acidimicrobidae	acidimicrobium	796
BPM1SH07	91	1	Proteobacteria	Beta	Burkholderiales	817
BPM1SH09	93	1	Proteobacteria	Beta	Burkholderiales	803
BPM1SH10	94	1	Proteobacteria	Gamma	γ-proteobacteria A	826
BPM1SH11	95	1	Actinobacteria	Corynebacterineae	Corynebacterium	806
BPM1SH12	96	1	Proteobacteria	Alpha	Acetobacteraceae	588

Table A-IV 31. Pennsylvania Mine Snowcover Long Band: ARB Divisions

ARB Name	RFLP type	Number of RFLPs	ARB division	ARB subdivision		Bases
BPM1LA01	1	2	Eucaryotes	environmental		450
BPM1LA02	2	33	Eucaryotes	Heterokonta		728
BPM1LB02	3	1	Proteobacteria	Gamma	Xanthomonadales	663
BPM1LG02	6	1	Cyanobacteria	chloroplasts		600
BPM1LH01	7	1	Eucaryotes	Heterokonta		625
BPM1LH02	8	1	Eucaryotes	Heterokonta		948
BPM1LB03	10	2	Cyanobacteria	chloroplasts		679
BPM1LE03	14	1	Chlamydiae	environmental		568
BPM1LF03	15	2	Firmicutes	bacilli	staphylococcaceae	746
BPM1LF04	16	2	Firmicutes	bacilli	staphylococcaceae	
BPM1LG11	17	3	Actinobacteria	Acidimicrobidae	acidimicrobium	751
BPM1LD10	20	2	Cyanobacteria	chloroplasts		723
BPM1LE06	22	1	Proteobacteria	Gamma	Xanthomonadales	714
BPM1LH05	24	1	Proteobacteria	Beta	environmental	695
BPM1LA12	27	2	Eucaryotes	Heterokonta		461
BPM1LC09	29	1	Cyanobacteria	chloroplasts		590
BPM1LD09	30	1	Eucaryotes	Heterokonta		562
BPM1LG10	33	1	Cyanobacteria	chloroplasts		771
BPM1LB11	35	1	Proteobacteria	Gamma	Xanthomonadales	780

Table A-IV 32. Pennsylvania Mine Snowcover Short Band: Best BLAST Matches

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BPM1SA01	D83363	1503	99	Staphylococcus epidermidis (strain ATCC 14990T) gene.16S rRNA
BPM1SA03	AB097215	1622	99	Propionibacterium acnes gene for 16S rRNA, complete sequence
BPM1SA04	D86510	1312	95	Acidocella sp. DNA for 16S rRNA
BPM1SA06	AY050601	1443	98	Uncultured bacterium clone GOUTB18 16S rRNA partial
BPM1SA07	AJ292583	1330	95	uncultured eubacterium WD257 partial 16S rRNA clone WD257
BPM1SA08	AF523917	1513	98	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM1SA09	AF523917	1453	98	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM1SA10	AF376025	1499	99	Frateuria sp. NO-16 16S rRNA, partial
BPM1SA11	D86510	1304	95	Acidocella sp. DNA for 16S rRNA
BPM1SB01	AJ519658	862	88	Uncultured gamma proteobacterium partial 16S rRNA clone
BPM1SB03	AF376025	1614	99	Frateuria sp. NO-16 16S rRNA, partial
BPM1SB04	AF523917	1475	98	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM1SB06	AF376025	1566	99	Frateuria sp. NO-16 16S rRNA, partial
BPM1SB07	D86510	1334	95	Acidocella sp. DNA for 16S rRNA
BPM1SB08	AF376025	1546	99	Frateuria sp. NO-16 16S rRNA, partial
BPM1SB09	AF376021	1586	99	Acidocella sp. NO-12 16S rRNA, partial
BPM1SB11	AJ292578	1376	97	uncultured eubacterium WD228 partial 16S rRNA clone WD228
BPM1SB12	AY050601	1495	98	Uncultured bacterium clone GOUTB18 16S rRNA partial
BPM1SC01	AF523917.1	1489	98	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM1SC05	X92696	1116	91	Actinomyces species 16S ribosomal RNA (isolate TM62)
BPM1SC06	AB097215	1622	100	Propionibacterium acnes gene for 16S rRNA, complete sequence
BPM1SC11	AF376025	1568	99	Frateuria sp. NO-16 16S rRNA, partial
BPM1SD01	AY082479	1049	91	Uncultured beta proteobacterium clone 44a-U1-9 16S rRNA
BPM1SD05	AF353297	1433	97	Uncultured bacterium Tui3-12 16S rRNA partial
BPM1SD06	AJ318183	1384	96	Uncultured gamma proteobacterium 16S rRNA clone BIsiii14
BPM1SD07	AF523917	1550	99	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM1SD12	AF289158	1306	95	Unidentified rhodophyte PRD01a010B 16S rRNA, partial
BPM1SE05	AF376025	1624	99	Frateuria sp. NO-16 16S rRNA, partial
BPM1SE06	AF376025	1586	99	Frateuria sp. NO-16 16S rRNA, partial
BPM1SE11	AF514855	1409	97	Haslea wawrikae 16S rRNA, partial; chloroplast
BPM1SE12	AY050601	1518	98	Uncultured bacterium clone GOUTB18 16S rRNA partial
BPM1SF01	AF376025	1590	99	Frateuria sp. NO-16 16S rRNA, partial
BPM1SF03	AJ292583	1271	95	uncultured eubacterium WD257 partial 16S rRNA clone WD257
BPM1SF06	AF376025	1566	99	Frateuria sp. NO-16 16S rRNA, partial
BPM1SF07	AJ292684	634	93	uncultured eubacterium WD272 partial 16S rRNA clone WD272
BPM1SF10	AJ401128	1275	94	Uncultured verrucomicrobium DEV020 partial 16S rRNA clone
BPM1SF11	AF523917	1522	98	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM1SF12	AF376021	1560	98	Acidocella sp. NO-12 16S rRNA, partial
BPM1SG01	D86510	1326	95	Acidocella sp. DNA for 16S rRNA
BPM1SG02	AJ292682	1281	94	uncultured eubacterium WD287 partial 16S rRNA clone WD287
BPM1SG04	AF376025	1610	99	Frateuria sp. NO-16 16S rRNA, partial
BPM1SG05	X92701	1568	98	Actinomyces species 16S ribosomal RNA (isolate TM177)
BPM1SG06	AF289158	1070	96	Unidentified rhodophyte PRD01a010B 16S rRNA, partial
BPM1SG07	AJ003058	1582	99	Propionibacterium propionicus DSM 43307 16S rRNA gene
BPM1SG08	AF376025	1564	98	Frateuria sp. NO-16 16S rRNA, partial

Table A-IV 32. Pennsylvania Mine Snowcover Short Band: Best BLAST Matches, continued

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BPM1SG09	AB097215	1639	100	Propionibacterium acnes gene for 16S rRNA, complete sequence
BPM1SG10	AF523917	1487	98	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM1SG12	AE003412	38	100	Drosophila melanogaster, chromosome 2L, region 34C4-36A7
BPM1SH03	AF376025	1600	99	Frateuria sp. NO-16 16S rRNA, partial
BPM1SH04	AJ401128	1229	94	Uncultured verrucomicrobium DEV020 partial 16S rRNA clone
BPM1SH05	AF376021	1542	99	Acidocella sp. NO-12 16S rRNA, partial
BPM1SH06	AF523917	1499	98	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM1SH07	AY050601	1485	97	Uncultured bacterium clone GOUTB18 16S rRNA partial
BPM1SH09	AY082479	1041	91	Uncultured beta proteobacterium clone 44a-U1-9 16S rRNA
BPM1SH10	U64034	1277	94	Legionella-like amoebal pathogen 1 16S rRNA, partial

Table A-IV 33. Pennsylvania Mine Snowcover Long Band: Best BLAST Matches

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BPM1LA01	AY544729	718	97	Crinula caliciiformis isolate AFTOL 272 18S rRNA,
BPM1LA02	AF123297	1372	98	Chrysolepidomonas dendrolepidota small subunit rRNA,
BPM1LB02	AB074730	1065	96	Gamma proteobacterium S-N(1)-6B 16S rRNA, partial
BPM1LG02			98	Asterionellopsis glacialis chloroplast 16S rRNA strain p126
BPM1LH01	AY180010	1094	97	Uncultured chrysophyte 18S small subunit rRNA, partial
BPM1LH02	AY180010	1689	97	Uncultured chrysophyte 18S small subunit rRNA, partial
BPM1LB03			95	Unidentified rhodophyte PRD01a010B 16S rRNA, partial
BPM1LE03	AY193206	866	94	Uncultured Chlamydiales bacterium 16S rRNA, partial
BPM1LF03	AY218744	1053	98	Uncultured bacterium clone KD5-94 16S rRNA, partial
BPM1LF04	AY587794	1308	98	Bacterium Te66A 16S rRNA, partial
BPM1LG11	AF523917	1154	98	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM1LD10			92	Uncultured soil bacterium PBS-22 partial 16S rRNA gene
BPM1LE06	AY495957	1259	98	Frateuria sp. WJ64 16S rRNA, partial
BPM1LH05	AF353297	1235	97	Uncultured bacterium Tui3-12 16S rRNA, partial
BPM1LA12	AF123297	850	98	Chrysolepidomonas dendrolepidota small subunit rRNA,
BPM1LC09			97	Acidocella sp. NO-12 16S rRNA, partial
BPM1LD09	AF123297	1003	97	Chrysolepidomonas dendrolepidota small subunit rRNA,
BPM1LG10			95	Unidentified rhodophyte PRD01a010B 16S rRNA, partial
BPM1LB11	AY218596	1298	96	Uncultured bacterium clone KD2-87 16S rRNA, partial

Table A-IV 34. Pennsylvania Mine Snowmelt: Best BLAST Matches for Partials

ARB Name	RFLP type	Accession Number	Bit Score	% ID	Best Blast Match
BPM2_E04	17	AF364574	38	100	Uncultured Chlamydiales bacterium clone P-8 16S rRNA,
BPM2_H08	34	AF323774	161	88	Uncultured bacterium clone BA068 16S rRNA, complete

Table A-IV 35. Pennsylvania Mine Snowcover Short Band: Best BLAST Matches for Partials

ARB Name	RFLP type	Accession Number	Bit Score	% ID	Best Blast Match
BPM1SH11	95	AF115947	1568	99	Uncultured Corynebacterium sp. MTcory19R 16S rRNA,
BPM1SH12	96	D86510	944	95	Acidocella sp. DNA for 16S rRNA
BPM1SA02	2	AJ309523	868	99	Red Sea bacterium KT-2K12 16S rRNA gene
BPM1SA05	5	AF376021	1007	98	Acidocella sp. NO-12 16S rRNA, partial
BPM1SA12	12	AF543499	642	92	Uncultured bacterium clone ASL8 16S rRNA, partial
BPM1SB05	17	AF523917	494	96	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM1SB10	22	AY123803	543	95	Nitrosovibrio tenuis 16S rRNA, partial
BPM1SC03	27	X97098	882	98	Unclassified Acidobacterium capsulatum phylum 16S rRNA
BPM1SC04	28	AY050601	587	97	Uncultured bacterium clone GOUTB18 16S rRNA partial
BPM1SC07	31	AY133431	24	100	Uncultured bacterium clone OB3_73 16S rRNA, partial
BPM1SC08	32	AP004438	34	100	Indostomus paradoxus mitochondrial DNA, genome
BPM1SC09	33	AF376021	920	98	Acidocella sp. NO-12 16S rRNA, partial
BPM1SC10	34	AJ292684	613	93	uncultured eubacterium partial 16S rRNA clone WD272
BPM1SC12	36	AF353297	712	97	Uncultured bacterium Tui3-12 16S rRNA, partial
BPM1SD02	38	X92700	710	99	Actinomyces species 16S ribosomal RNA (isolate TM167)
BPM1SD03	39	AJ289984	652	93	Uncultured bacterium FukuN108 16S rRNA gene
BPM1SD04	40	X92700	979	98	Actinomyces species 16S ribosomal RNA (isolate TM167)
BPM1SD08	44	AF376021	842	97	Acidocella sp. NO-12 16S rRNA, partial
BPM1SD09	45	AF376021	920	98	Acidocella sp. NO-12 16S rRNA, partial
BPM1SD10	46	AF523917	910	97	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM1SD11	47	AB074730	926	98	Gamma proteobacterium S-N(1)-6B gene 16S rRNA, partial
BPM1SE01	49	AB097215	442	99	Propionibacterium acnes gene 16S rRNA, complete sequence
BPM1SE02	50	X92700	961	99	Actinomyces species 16S ribosomal RNA (isolate TM167)
BPM1SE03	51	AJ292578	454	96	uncultured eubacterium partial 16S rRNA clone WD228
BPM1SE04	52	AB087322	902	98	Uncultured bacterium gene for 16S rRNA, partial,
BPM1SE07	55	AJ292684	803	96	uncultured eubacterium partial 16S rRNA clone WD272
BPM1SE08	56	AF353293	591	98	Uncultured bacterium Tui2-30 16S rRNA, partial
BPM1SE10	58	AF523917	559	95	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM1SF02	62	AB075126	722	99	Uncultured bacterium gene for 16S rRNA, partial,
BPM1SF04	64	AF353297	888	97	Uncultured bacterium Tui3-12 16S rRNA partial
BPM1SF08	68	AF523917	916	99	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM1SG03	75	X92700	900	99	Actinomyces species 16S ribosomal RNA (isolate TM167)
BPM1SH02	86	AF376021	515	95	Acidocella sp. NO-12 16S rRNA, partial
BPM1SH08	92	AF523917	967	94	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM1SH01	85	AB097215	626	99	Propionibacterium acnes 6S rRNA, complete sequence

Table A-IV 36. Pennsylvania Mine Snowcover Long Band: Best BLAST Matches for Partials

ARB Name	RFLP type	Accession Number	Bit Score	% ID	Best Blast Match
BPM1LA04	9	AF351579	706	95	Cryptocaryon irritans small subunit ribosomal RNA, partial
BPM1LB04	11	AF122885	1503	97	Legionella pneumophila subsp. pascullei 16S rRNA,
BPM1LG08	12	AY180010	934	97	Uncultured chrysophyte 18S small subunit rRNA, partial
BPM1LD03	13	AF376021	1503	98	Acidocella sp. NO-12 16S rRNA, partial
BPM1LA05	18	AY495957	1441	99	Frateuria sp. WJ64 16S rRNA, partial
BPM1LA06	19	AF523902	1465	99	Uncultured bacterium clone RCP2-13 16S rRNA, partial
BPM1LF11	21	AF523917	1400	98	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM1LF06	23	AB074730	1187	98	Gamma proteobacterium S-N(1)-6B gene for 16S rRNA, partial
BPM1LH06	25	AY096034	1128	98	Acidobacterium sp. WJ7 16S rRNA, partial
BPM1LC07	26	AY495957	1287	99	Frateuria sp. WJ64 16S rRNA, partial
BPM1LF10	31	AJ536876	698	95	Uncultured bacterium partial 16S rRNA clone JG30a-KF-32
BPM1LG09	32	AF122885	987	97	Legionella pneumophila subsp. pascullei 16S rRNA,
BPM1LE08	28	AF401527	1158	96	Epistylis galea small subunit rRNA, complete sequence
BPM1LH10	34	AY180010	948	97	Uncultured chrysophyte 18S small subunit rRNA, partial
BPM1LE12	36	AY180010	1156	97	Uncultured chrysophyte 18S small subunit rRNA, partial
BPM1LD04	4	AY124367	1614	99	Uncultured metazoan clone CSE21 small subunit rRNA,
BPM1LD02	5	AJ292578	914	96	uncultured eubacterium partial 16S rRNA clone WD228
BPM1LH11	37	AY180010	963	97	Uncultured chrysophyte 18S small subunit rRNA, partial

Table A-IV 38. Pennsylvania Mine Snowmelt: Best BLAST Matches

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BPM2_A01	AF523917	1497	98	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM2_H01	AJ292609	1298	95	uncultured eubacterium partial 16S rRNA clone WD2105
BPM2_B02	AF376025	1570	99	Frateuria sp. NO-16 16S rRNA, partial
BPM2_F10	AY050601	1546	98	Uncultured bacterium clone GOUTB18 16S rRNA, partial
BPM2_C02	AF289158	946	96	Unidentified rhodophyte PRD01a010B 16S rRNA, partial
BPM2_D01	AF534192	1473	97	Uncultured bacterium clone Glu1 16S rRNA, partial
BPM2_E01	AJ292579	1386	97	uncultured eubacterium partial 16S rRNA clone WD243
BPM2_F01	AF523878	103	85	Uncultured bacterium clone RCP2-17 16S rRNA, partial
BPM2_G01	AF498683	1491	98	Bacterium Ellin301 16S rRNA, partial
BPM2_H02	AJ292578	1489	98	uncultured eubacterium partial 16S rRNA clone WD228
BPM2_A03	AF407387	1433	97	Uncultured bacterium clone RB7C10 16S rRNA, partial
BPM2_A04	AY050601	1491	98	Uncultured bacterium clone GOUTB18 16S rRNA, partial
BPM2_B03	AJ276901	1116	92	Hymenobacter aerophilus 16S rRNA strain I/26Cor1
BPM2_C03	AF523947	1118	96	Uncultured bacterium clone FW94 16S rRNA, partial
BPM2_D03	U62836	904	92	Unidentified eubacterium RB13 16S rRNA, partial
BPM2_E03	AJ440237	414	86	Conexibacter woesei partial 16S rRNA strain DSM 14684T
BPM2_F03	AF225446	1322	95	Uncultured bacterium BA18 16S rRNA, partial
BPM2_H03	AJ306776	638	86	Uncultured bacterium partial 16S rRNA clone SHA-117
BPM2_A05	U24580	1037	91	Angiopteris evecta chloroplast 16S rRNA partial
BPM2_A06	AJ519399	1568	98	Uncultured actinobacterium partial 16S rRNA JG37-AG-83
BPM2_C06	AF376024	1497	97	Acidisphaera sp. NO-15 16S rRNA, partial
BPM2_D06	D30776	1645	99	Acidiphilium rubrum gene for 16S ribosomal RNA
BPM2_E06	AJ290012	1556	99	Uncultured bacterium FukuS27 16S rRNA gene
BPM2_F06	AJ292578	1487	98	uncultured eubacterium partial 16S rRNA clone WD228
BPM2_G05	AF465657	1076	92	Uncultured planctomycete YNPRH54A 16S rRNA, partial
BPM2_H05	AF289152	860	90	Uncultured bacterium clone PRD01a004B 16S rRNA partial
BPM2_H06	AF050573	1566	98	Uncultured eubacterium WCHB1-08 16S rRNA, partial
BPM2_B08	AY048891	1497	98	Uncultured bacterium Q3-24C2 16S rRNA partial
BPM2_D07	AJ292684	1013	90	uncultured eubacterium partial 16S rRNA clone WD272
BPM2_F08	AJ292578	1455	97	uncultured eubacterium partial 16S rRNA clone WD228
BPM2_G08	AB005875	363	98	Unidentified bacterium 16S rRNA, partial
BPM2_H07	AF512827	1431	96	Burkholderia sordicola strain SNU 020123 16S rRNA,
BPM2_A09	AF465658	1308	95	Uncultured Acidobacterium group YNPRH5A 16S rRNA
BPM2_A10	AB010909	1556	99	Gordona sp. gene for 16S rRNA, strain:SJS0289-JS1
BPM2_B10	AF376025	1495	97	Frateuria sp. NO-16 16S rRNA, partial
BPM2_D10	AJ519409	1386	97	Uncultured bacterium partial 16S rRNA JG37-AG-138
BPM2_H10	AF446273	161	83	Unidentified eukaryote FL07F11 16S rRNA, partial
BPM2_B11	AJ306790	367	91	Uncultured bacterium partial 16S rRNA clone SHA-59
BPM2_C11	AF376025	1604	99	Frateuria sp. NO-16 16S rRNA, partial
BPM2_C12	AF376025	1001	96	Frateuria sp. NO-16 16S rRNA, partial
BPM2_E12	X68415	1633	99	B.globisporus gene for 16S rRNA
BPM2_G11	AF225446	1324	95	Uncultured bacterium BA18 16S rRNA, partial
BPM2_G12	AF376025	1522	98	Frateuria sp. NO-16 16S rRNA, partial
BPM2_H12	AF376025	1556	98	Frateuria sp. NO-16 16S rRNA, partial
BPM2_B07	AJ318183	1429	96	Uncultured gamma proteobacterium 16S rRNA, BIsiii14

Table A-IV 39. Pennsylvania Mine Summer: ARB Divisions

ARB Name	RFLP type	Number of RFLPs	ARB Division	ARB Subdivision		Bases
BPM3_A01	1	1	Proteobacteria	Beta	Burkholderiales	816
BPM3_B01	3	1	Proteobacteria	Delta	environmental	814
BPM3_C01	4	2	Acidobacteria	environmental		805
BPM3_C02	5	2	Proteobacteria	Delta	environmental	843
BPM3_D01	6	1	Proteobacteria	Alpha	Acetobacteraceae	824
BPM3_D02	7	1	Actinobacteria	Microbacteriaceae	environmental	809
BPM3_E01	8	1	Proteobacteria	Gamma	Xanthomonadales	664
BPM3_E02	9	1	Proteobacteria	Gamma	Environ. clone group	833
BPM3_F01	10	4	Proteobacteria	Beta	environmental	829
BPM3_F02	11	1	Verrucomicrobia	VER-3	environmental	840
BPM3_G01	12	15	Proteobacteria	Gamma	Xanthomonadales	840
BPM3_H01	13	1	Chloroflexi	ARD environmental group		648
BPM3_A03	14	1	Acidobacteria	Acidobacteria-1	Acap group	844
BPM3_A04	15	6	Proteobacteria	Gamma	Xanthomonadales	841
BPM3_B03	16	2	Proteobacteria	Gamma	Xanthomonadales	819
BPM3_B04	17	1	Proteobacteria	Beta	environmental	810
BPM3_A07	18	12	Proteobacteria	Beta	Burkholderiales	822
BPM3_B10	19	2	Cyanobacteria	chloroplasts		847
BPM3_A05	22	1	Actinobacteria	Acidimicrobidae	acidimicrobium	805
BPM3_B06	23	1	Proteobacteria	Alpha	environmental	839
BPM3_C05	24	2	Proteobacteria	Beta	Burkholderiales	840
BPM3_C06	25	1	Proteobacteria	Gamma	Xanthomonadales	848
BPM3_F06	27	4	Proteobacteria	Alpha	Acetobacteraceae	817
BPM3_E06	28	1	Chlamydiae	endosymbionts of Acanthamoeba sp.		826
BPM3_G05	29	1	Proteobacteria	Delta	environmental	822
BPM3_H05	30	2	Proteobacteria	Alpha	environmental	810
BPM3_C07	31	1	Proteobacteria	Alpha	Acetobacteraceae	780
BPM3_G07	32	1	Acidobacteria	Acidobacteria-1	environmental	826
BPM3_B11	33	2	Proteobacteria	Gamma	Environ. clone group	860
BPM3_G08	33	2	Actinobacteria	Acidimicrobidae	acidimicrobium	799
BPM3_A09	34	1	Proteobacteria	Alpha	Rhickettsiales	827
BPM3_B12	36	2	Acidobacteria	Acidobacteria-1	Acap group	1512
BPM3_F10	38	1	Chloroflexi	ARD environmental group		825
BPM3_G10	39	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	808
BPM3_D11	40	1	Chloroflexi	ARD environmental group		830
BPM3_D12	41	1	Actinobacteria	Acidimicrobidae	acidimicrobium	821
BPM3_G12	42	1	Proteobacteria	Gamma	Xanthomonadales	851

Table A-IV 40. Pennsylvania Mine Summer: Best BLAST Matches

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BPM3_A01	AY082479	1039	91	Uncultured beta proteobacterium clone 44a-U1-9 16S rRNA
BPM3_B01	AF523886	1350	96	Uncultured bacterium clone RCP2-54 16S rRNA, partial
BPM3_C01	AJ292578	1429	97	uncultured eubacterium partial 16S rRNA clone WD228
BPM3_C02	AF543496	1336	95	Uncultured bacterium clone AS6 16S rRNA, partial
BPM3_D01	D30769	1227	94	Acidiphilium sp. gene for 16S ribosomal RNA
BPM3_D02	AF050573	1548	99	Uncultured eubacterium WCHB1-08 16S rRNA, partial
BPM3_E01	AJ318183	1144	96	Uncultured gamma proteobacterium 16S rRNA BIsiii14
BPM3_E02	AJ292673	1534	98	uncultured eubacterium partial 16S rRNA clone WD260
BPM3_F01	AF353297	1465	97	Uncultured bacterium Tui3-12 16S rRNA partial
BPM3_F02	AJ401128	1257	94	Uncultured verrucomicrobium DEV020 partial 16S rRNA
BPM3_G01	AF376025	1598	98	Frateuria sp. NO-16 16S rRNA, partial
BPM3_H01	AF254398	291	85	Uncultured bacterium UASB_TL14 16S rRNA, complete
BPM3_A03	AJ292583	1304	95	uncultured eubacterium partial 16S rRNA clone WD257
BPM3_A04	AJ318183	1378	95	Uncultured gamma proteobacterium 16S rRNA BIsiii14
BPM3_B03	AF376025	1548	99	Frateuria sp. NO-16 16S rRNA, partial
BPM3_B04	AF351236	1457	97	Uncultured beta proteobacterium clone 8-5 16S rRNA,
BPM3_A07	AY050601	1465	97	Uncultured bacterium clone GOUTB18 16S rRNA partial
BPM3_B10	X75522	1602	98	K.flaccidum plastid 16S rRNA gene
BPM3_A05	AF523917	1485	98	Uncultured bacterium clone RCP2-103 16S rRNA, partial
BPM3_B06	AF236003	1407	96	Alpha proteobacterium A0902 16S rRNA partial
BPM3_C05	AF407387	1503	97	Uncultured bacterium clone RB7C10 16S rRNA, partial
BPM3_C06	AF376025	1620	99	Frateuria sp. NO-16 16S rRNA, partial
BPM3_F06	AF376021	1572	99	Acidocella sp. NO-12 16S rRNA, partial
BPM3_E06	AF083614	1243	94	Endosymbiont of Acanthamoeba sp. UWE1 16S rRNA
BPM3_G05	AF543496	1332	95	Uncultured bacterium clone AS6 16S rRNA, partial
BPM3_H05	Y07582	1423	97	Uncultured bacterium DA067 16S rRNA gene
BPM3_C07	AF523875	1330	97	Uncultured bacterium clone RCP2-10 16S rRNA, partial
BPM3_G07	AF498692	1338	95	Bacterium Ellin310 16S rRNA, partial
BPM3_B11	AJ292673	1558	98	uncultured eubacterium partial 16S rRNA clone WD260
BPM3_G08	AF523916	1542	99	Uncultured bacterium clone RCP2-2 16S rRNA, partial
BPM3_A09	L36106	884	88	Rickettsia massiliae 16S ribosomal RNA (16S rRNA) gene
BPM3_B12	AF498733	1362	96	Bacterium Ellin351 16S rRNA, partial
BPM3_F10	AF465668	422	92	Uncultured bacterium DGGE band YNPRH-B10 16S rRNA
BPM3_G10	AF289158	1310	95	Unidentified rhodophyte PRD01a010B 16S rRNA, partial
BPM3_D11	AF465668	367	90	Uncultured bacterium DGGE band YNPRH-B10 16S rRNA
BPM3_D12	X92703	1501	98	Actinomyces species 16S ribosomal RNA (isolate TM208)
BPM3_G12	AJ318183	1396	95	Uncultured gamma proteobacterium 16S rRNA BIsiii14

Table A-IV 41. Pennsylvania Mine Summer: Best BLAST Matches for Partials

ARB Name	RFLP type	Accession Number	Bit Score	% ID	Best Blast Match
BPM3_E03	20	AF059758	648	95	Uncultured eubacterium DgEPI2 16S rRNA, partial
BPM3_G04	21	AF391981	240	93	Uncultured thermal soil bacterium clone YNPFFP40 16S rRNA
BPM3_D10	37	AF289910	69	94	Uncultured alpha proteobacterium Kmlps6-15 16S rRNA,
BPM3_G04	21	AY050601	561	96	Uncultured bacterium clone GOUTB18 16S rRNA partial

Table A-IV 42. Peru Creek Snowcover: ARB Divisions

ARB Name	RFLP Type	Number of RFLPs	ARB Division	ARB Subdivision		Bases
BPC1_A02	2	1	ARD1	environmental		804
BPC1_B01	3	47	Acidobacteria	Acidobacteria-3	environmental	800
BPC1_B07	4	2	Proteobacteria	Beta	Burkholderiales	852
BPC1_C02	5	1	Bacteroidetes	Saprospiraceae	environmental	844
BPC1_D07	5	2	Proteobacteria	Alpha	Bradyrhizobiaceae	805
BPC1_A06	6	6	Acidobacteria	Acidobacteria-1	Acap group	825
BPC1_F02	7	1	Acidobacteria	environmental		841
BPC1_H05	8	2	Acidobacteria	Acidobacteria-3	environmental	848
BPC1_D08	10	4	Proteobacteria	Beta		812
BPC1_A03	10	1	Proteobacteria	Beta	Methylophilus.	815
BPC1_A05	11	2	Proteobacteria	Gamma	Xanthomonadales	848
BPC1_D04	12	3	bacterial environmental samples			791
BPC1_G04	13	1	Cyanobacteria	environmental		869
BPC1_H03	14	1	Proteobacteria	Beta	environmental	801
BPC1_D05	15	3	Acidobacteria	ARD environmental group		847
BPC1_E05	16	1	Proteobacteria	Alpha	environmental	841
BPC1_E06	17	2	Acidobacteria	ARD environmental group		831
BPC1_H06	19	1	Actinobacteria	Cellulomonadaceae	environmental	868
BPC1_B08	20	1	Proteobacteria	Alpha	environmental	840
BPC1_A09	22	1	Acidobacteria	Acidobacteria-3	environmental	822
BPC1_E09	23	2	Bacteroidetes	Sphingobacteriaceae	environmental	835

Table A-IV 43. Peru Creek Snowcover: Best BLAST Matches for Partials

ARB Name	RFLP Type	Accession Number	Bit Score	% ID	Best Blast Match
BPC1_F05	18	AJ536865	1302	98	Uncultured bacterium partial 16S rRNA clone JG30-KF-A11
BPC1_A01	1	AJ290177	396	96	Uncultured planctomycete partial 16S rRNA clone DSP01
BPC1_H09	9	AJ519380	997	97	Uncultured Holophaga sp. partial 16S rRNA clone JG37-AG-74
BPC1_H10	9	U62843	866	93	Unidentified eubacterium RB22 16S rRNA, partial
BPC1_C07	21	AJ229177	498	96	Unidentified eubacterium from anoxic bulk soil 16S rRNA gene
BPC1_G11	25	AY171339	751	98	Uncultured bacterium clone s27 16S rRNA, partial

Table A-IV 44. Peru Creek Snowcover: Best BLAST Matches

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BPC1_A02	AJ519409	1388	97	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC1_B01	AJ519380	1384	97	Uncultured Holophaga sp. partial 16S rRNA clone JG37-AG-74
BPC1_B07	AJ505855	1318	95	Comamonadaceae bacterium PIV-3D partial 16S rRNA st.PIV-3D
BPC1_C02	AF527580	1207	93	Uncultured bacterium clone LPB08 16S rRNA, partial
BPC1_A06	AB089126	1421	96	Uncultured Acidobacteriaceae bacterium gene for 16S rRNA, partial
BPC1_F02	AF524859	1499	97	Bacterium K-5b10 16S rRNA, partial
BPC1_H05	AJ519380	1447	96	Uncultured Holophaga sp. partial 16S rRNA clone JG37-AG-74
BPC1_D08	AJ421928	1471	97	Uncultured beta proteobacterium partial 16S rRNA clone Elb168
BPC1_A03	AJ532688	1495	98	Uncultured beta proteobacterium partial 16S rRNA clone
BPC1_A05	AF376025	1588	98	Frateuria sp. NO-16 16S rRNA, partial
BPC1_D04	AJ536865	1489	98	Uncultured bacterium partial 16S rRNA clone JG30-KF-A11
BPC1_G04	AB042972	1633	99	Phormidium tenue (strain:C) gene for 16S rRNA, partial
BPC1_H03	AJ421936	1265	95	Uncultured beta proteobacterium partial 16S rRNA clone Elb236
BPC1_D05	AJ519372	1584	99	Uncultured Holophaga sp. partial 16S rRNA clone JG37-AG-47
BPC1_E05	AJ458470	1350	95	Methylocystis sp. IMET 10484 partial 16S rRNA strain IMET 10484
BPC1_E06	AJ519370	1298	94	Uncultured Holophaga sp. partial 16S rRNA clone JG37-AG-40
BPC1_H06	AB078822	1691	99	Cellulomonadaceae str. K8 gene for 16S ribosomal RNA, partial
BPC1_B08	AJ292592	1467	97	uncultured eubacterium WD225 partial 16S rRNA clone WD225
BPC1_A09	AJ519380	1423	96	Uncultured Holophaga sp. partial 16S rRNA clone JG37-AG-74
BPC1_E09	AJ295473	1435	96	Uncultured rape rhizosphere bacterium wr0012 partial 16S rRNA
BPC1_E11	U68632	420	96	Unidentified eubacterium from the Amazon 16S rRNA

Table A-IV 45. Peru Creek Summer: Best BLAST Matches for Partials

ARB Name	RFLP Type	Accession Number	Bit Score	% ID	Best Blast Match
BPC3_A03	17	AJ536871	1314	98	Uncultured gamma proteobacterium partial 16S rRNA clone
BPC3_F03	27	AJ536880	714	95	Uncultured bacterium partial 16S rRNA clone JG30-KF-AS41
BPC3_C05	37	AL596172	36	100	Listeria innocua Clip11262 complete genome, segment 10/12
BPC3_D05	39	AF417875	65	100	Uncultured antarctic soil bacterium clone bh1.36 16S rRNA
BPC3_D07	55	AY168738	81	95	Uncultured bacterium clone HC-29 16S rRNA, partial
BPC3_G10	78	AJ421928	1047	98	Uncultured beta proteobacterium partial 16S rRNA clone Elb168
BPC3_B12	84	Y08541	1235	97	M.multipartita 16S rRNA gene
BPC3_B02	4	U35000	541	93	Bradyrhizobium elkanii 16S rRNA, partial;
BPC3_H03	31	AJ532696	502	96	Uncultured gamma proteobacterium partial 16S rRNA clone
BPC3_D06	40	AJ292673	325	92	uncultured eubacterium WD260 partial 16S rRNA clone WD260
BPC3_B07	51	AJ534624	856	91	Uncultured proteobacterium partial 16S rRNA JG36-TzT-56
BPC3_F07	59	AE016802	34	100	Vibrio vulnificus CMCP6 chromosome I 6 of 11: complete

Table A-IV 46. Peru Creek Snowmelt: ARB Divisions

ARB Name	RFLP Type	Number of RFLPs	ARB Division	ARB subdivision		Bases
BPC2_A01	1	1	Proteobacteria	Beta	environmental	839
BPC2_A02	2	1	Proteobacteria	Beta	environmental	839
BPC2_B01	3	1	Bacteroidetes	Flavobacteriales	Sporocytophaga	827
BPC2_C01	5	1	Acidobacteria	ARD clone		840
BPC2_C02	6	1	Proteobacteria	Beta	Burkholderiales	841
BPC2_D01	7	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	806
BPC2_D02	8	1	Proteobacteria	Alpha	Bradyrhizobiaceae	844
BPC2_F02	12	1	Proteobacteria	Beta	Burkholderiales	823
BPC2_G01	13	1	Chloroflexi	ARD clone		813
BPC2_G02	14	1	Acidobacteria	ARD clone		854
BPC2_H01	15	1	ARD1	environmental		834
BPC2_H02	16	1	Chloroflexi	Chloroflexi-1	Chloroflexi 1b	867
BPC2_A03	17	1	Acidobacteria	Acidobacteria-1	environmental	819
BPC2_B04	20	1	ARD1	environmental		803
BPC2_C04	22	1	Proteobacteria	Beta	environmental	776
BPC2_E03	25	1	Actinobacteria	Acidimicrobidae	acidimicrobium	799
BPC2_E04	26	1	Proteobacteria	Beta	environmental	828
BPC2_F03	27	1	Acidobacteria	Acidobacteria-3	environmental	743
BPC2_G03	29	1	bacterial environmental samples			859
BPC2_G04	30	1	Proteobacteria	Alpha	DA122 soil group	830
BPC2_H03	31	1	Proteobacteria	Gamma	Environ. clone group	837
BPC2_H04	32	1	Proteobacteria	Alpha	environmental	844
BPC2_B05	35	1	Proteobacteria	Alpha	Acetobacteraceae	843
BPC2_D05	39	1	Proteobacteria	Alpha	environmental	811
BPC2_D06	40	1	ARD1	environmental		797
BPC2_E06	42	1	Proteobacteria	Delta	environmental	818
BPC2_G05	45	1	Proteobacteria	Alpha	environmental	795
BPC2_G06	46	1	Acidobacteria	ARD clone		848
BPC2_H05	47	1	ARD1	environmental		834
BPC2_H06	48	1	Chloroflexi	ARD clone		815
BPC2_A07	49	1	Actinobacteria	Acidimicrobidae	acidimicrobium	794
BPC2_A08	50	1	Actinobacteria	Acidimicrobidae	acidimicrobium	823
BPC2_B07	51	1	Acidobacteria	ARD clone		843
BPC2_B08	52	1	ARD1	environmental		829
BPC2_C07	53	1	Proteobacteria	Alpha	environmental	842
BPC2_C08	54	1	Actinobacteria	Acidimicrobidae	acidimicrobium	831
BPC2_D07	55	1	Proteobacteria	Delta	Desulfuromonadales	825
BPC2_D08	56	1	ARD1	environmental		792
BPC2_F08	60	1	ARD1	environmental		791
BPC2_G08	62	1	Proteobacteria	Beta	environmental	838
BPC2_H07	63	1	ARD1	environmental		815
BPC2_H08	64	1	Actinobacteria	Acidimicrobidae	acidimicrobium	818
BPC2_A09	65	1	Proteobacteria	Gamma	Environ. clone group	825
BPC2_A10	66	1	Bacteroidetes	Saprospiraceae	environmental	838

Table A-IV 46. Peru Creek Snowmelt: ARB Divisions, continued

ARB Name	RFLP Type	Number of RFLPs	ARB Division	ARB subdivision		Bases
BPC2_B09	67	1	Proteobacteria	Gamma	Environ. clone group	857
BPC2_B10	68	1	Proteobacteria	Beta	environmental	841
BPC2_C09	69	1	Acidobacteria	Acidobacteria-3	environmental	842
BPC2_D09	71	1	OP10	environmental		811
BPC2_D10	72	1	Acidobacteria	ARD clone		826
BPC2_E10	74	1	Proteobacteria	Gamma	Environ. clone group	823
BPC2_F10	76	1	Acidobacteria	ARD clone		837
BPC2_G09	77	1	Actinobacteria	Acidimicrobidae	acidimicrobium	826
BPC2_H09	79	1	Acidobacteria	ARD clone		830
BPC2_A11	81	1	Proteobacteria	Gamma	Environ. clone group	798
BPC2_B11	83	1	ARD1	environmental		788
BPC2_B12	84	1	ARD1	environmental		816
BPC2_C11	85	1	Acidobacteria	Acidobacteria-3	environmental	836
BPC2_C12	86	1	Cyanobacteria	chloroplasts	Chlamydomonas: Chloropla	837
BPC2_D11	87	1	Chloroflexi	ARD clone		832
BPC2_E11	89	1	Proteobacteria	Beta	environmental	835
BPC2_E12	90	1	Acidobacteria	ARD clone		848
BPC2_F12	92	1	Proteobacteria	Delta	Bdellovibrionales	832
BPC2_G12	94	1	Acidobacteria	Acidobacteria-3	environmental	840
BPC2_H11	95	1	ARD1	environmental		795
BPC2_H12	96	1	ARD1	environmental		814

Table A-IV 47. Peru Creek Snowmelt: Best BLAST Matches

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BPC2_A01	AF072922	1318	95	Uncultured bacterium S28 16S rRNA, partial
BPC2_A02	AF072922	1302	94	Uncultured bacterium S28 16S rRNA, partial
BPC2_B01	AF260716	1520	98	Cytophaga sp. P1 16S rRNA, partial
BPC2_C01	AJ534634	1471	98	Uncultured Acidobacterium group bacterium partial 16S rRNA
BPC2_C02	AF407387	1517	97	Uncultured bacterium clone RB7C10 16S rRNA, partial
BPC2_D01	U82327	442	91	Moorella glycerini 16S small subunit rRNA, complete
BPC2_D02	Z94805	1612	99	Bradyrhizobium genosp. P 16S rRNA gene
BPC2_F02	AF407387	1451	97	Uncultured bacterium clone RB7C10 16S rRNA, partial
BPC2_G01	AJ519409	369	85	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC2_G02	AJ292580	1263	94	uncultured eubacterium WD244 partial 16S rRNA clone WD244
BPC2_H01	AJ519409	1380	96	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC2_H02	AF234690	1413	95	Uncultured sludge bacterium H39 16S rRNA, partial
BPC2_A03	AF200699	1552	98	Uncultured Acidobacterium UA3 16S rRNA, partial
BPC2_B04	AJ519409	1314	96	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC2_C04	AF407387	1213	94	Uncultured bacterium clone RB7C10 16S rRNA, partial
BPC2_E03	X92695	1362	96	Actinomyces species 16S ribosomal RNA (isolate TM56)
BPC2_E04	AF407387	1451	97	Uncultured bacterium clone RB7C10 16S rRNA, partial
BPC2_F03	AJ519380	664	95	Uncultured Holophaga sp. partial 16S rRNA clone JG37-AG-74
BPC2_G03	AJ292675	761	90	uncultured eubacterium WD284 partial 16S rRNA clone WD284
BPC2_G04	AF523949	1495	97	Uncultured bacterium clone FW91 16S rRNA, partial

Table A-IV 47. Peru Creek Snowmelt: Best BLAST Matches, continued

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BPC2_H03	AB062831	938	89	uncultured bacterium gene for 16S rRNA, partial,
BPC2_H04	AF253412	1011	95	Acidocella sp. WJB-3 16S rRNA, partial
BPC2_B05	AJ292606	1477	97	uncultured eubacterium WD295 partial 16S rRNA clone WD295
BPC2_D05	AF361188	841	96	Caulobacter sp. A1 16S rRNA, partial
BPC2_D06	AJ519409	1368	96	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC2_E06	AF523892	890	97	Uncultured bacterium clone RCP1-43 16S rRNA, partial
BPC2_G05	AF236003	1364	96	Alpha proteobacterium A0902 16S rRNA, partial
BPC2_G06	AJ519372	1608	99	Uncultured Holophaga sp. partial 16S rRNA clone JG37-AG-47
BPC2_H05	AJ519409	1348	95	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC2_H06	AF465676	383	91	Uncultured bacterium DGGE band YNPRH-B18 16S rRNA,
BPC2_A07	AF523916.	1455	98	Uncultured bacterium clone RCP2-2 16S rRNA, partial
BPC2_A08	X97098	912	98	Unclassified Acidobacterium capsulatum phylum 16S rRNA
BPC2_B07	AJ519372	910	98	Uncultured Holophaga sp. partial 16S rRNA clone JG37-AG-47
BPC2_B08	AJ519409	1402	97	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC2_C07	Y07582	1469	97	Uncultured bacterium DA067 16S rRNA gene
BPC2_C08	X97098	928	98	Unclassified Acidobacterium capsulatum phylum16S rRNA
BPC2_D07	Y19190	1487	97	Geobacter sp. 16S rRNA strain CdA-2
BPC2_D08	AJ295657	823	96	Uncultured bacterium KF/GS-JG36-31 partial 16S rRNA gene
BPC2_F08	AJ519409	1330	96	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC2_G08	AF072922	1292	94	Uncultured bacterium S28 16S rRNA, partial
BPC2_H07	AJ519409	1392	98	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC2_H08	X92700	1041	96	Actinomyces species 16S ribosomal RNA (isolate TM167)
BPC2_A09	AJ292673	1556	98	uncultured eubacterium WD260 partial 16S rRNA clone WD260
BPC2_A10	AJ318106	1314	94	Uncultured bacterium 16S rRNA clone BIai2
BPC2_B09	AJ292673	1580	98	uncultured eubacterium WD260 partial 16S rRNA clone WD260
BPC2_B10	AF351229	1209	93	Uncultured beta proteobacterium clone 4-21 16S rRNA,
BPC2_C09	AF523987	1185	94	Uncultured bacterium clone FW112 16S rRNA, partial
BPC2_D09	AJ244807	1211	93	Gram-positive bacteria SOGA31 partial 16S rRNA gene
BPC2_D10	AJ292580	1235	94	uncultured eubacterium WD244 partial 16S rRNA clone WD244
BPC2_E10	AJ292676	1285	94	uncultured eubacterium WD2124 partial 16S rRNA clone WD2124
BPC2_F10	AJ295657	1431	96	Uncultured bacterium KF/GS-JG36-31 partial 16S rRNA gene
BPC2_G09	Z73390	1148	95	Actinomycete species 16S rRNA gene (clone Ep_T1.217)
BPC2_H09	AJ295657	1439	96	Uncultured bacterium KF/GS-JG36-31 partial 16S rRNA gene
BPC2_A11	AJ518761	1183	96	Uncultured alpha proteobacterium partial 16S rRNA clone
BPC2_B11	AJ519409	1356	97	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC2_B12	AJ519409	1423	97	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC2_C11	AF498753	916	95	Bacterium Ellin371 16S rRNA, partial
BPC2_C12	X75522	1469	97	K.flaccidum plastid 16S rRNA gene
BPC2_D11	AY050601	571	96	Uncultured bacterium clone GOUTB18 16S rRNA, partial
BPC2_E11	AL646073	40	100	Ralstonia solanacearum GMI1000 chromosome, complete sequence
BPC2_E12	AJ252633	1463	97	Agricultural soil bacterium clone SC-I-39, 16S rRNA gene (partial)
BPC2_F12	AF084851	1055	93	Bdellovibrio bacteriovorus strain E 16S rRNA, partial
BPC2_G12	AJ519380	1459	97	Uncultured Holophaga sp. partial 16S rRNA clone JG37-AG-74
BPC2_H11	AJ519409	1144	93	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC2_H12	AJ519409	1372	97	Uncultured bacterium partial 16S rRNA clone JG37-AG-138

Table A-IV 48. Peru Creek Snowmelt: Best BLAST Matches for Partials

ARB Name	RFLP Type	Accession Number	Bit Score	% ID	Best Blast Match
BPC2_F01	11	AF429182	412	92	Uncultured bacterium clone CR99-24-72 16S rRNA,
BPC2_A06	34	AF465676	359	91	Uncultured bacterium DGGE band YNPRH-B18 16S rRNA,
BPC2_B06	36	U62855	773	98	Unidentified eubacterium RB38 16S rRNA, partial
BPC2_E05	41	AY143789	611	97	Uncultured Rubrobacteridae bacterium clone Vr33 16S rRNA
BPC2_C10	70	AJ518790	182	91	Uncultured delta proteobacterium partial 16S rRNA clone
BPC2_G10	78	AJ295657	1116	97	Uncultured bacterium KF/GS-JG36-31 partial 16S rRNA gene
BPC2_G11	93	AJ518761	1122	98	Uncultured alpha proteobacterium partial 16S rRNA clone
BPC2_B02	4	AF200698	513	97	Uncultured Acidobacterium UA2 16S rRNA, partial
BPC2_E01	9	AF498722	319	86	Bacterium Ellin340 16S rRNA, partial
BPC2_E02	10	AY133069	272	92	Uncultured beta proteobacterium clone ccs214 16S rRNA
BPC2_B03	19	AJ519378	741	95	Uncultured Holophaga sp. partial 16S rRNA clone JG37-AG-67
BPC2_D04	24	AJ271048	781	93	uncultured bacterium GC55 partial 16S rRNA gene
BPC2_F04	28	AJ252651	192	93	Agricultural soil bacterium clone SC-I-71, 16S rRNA gene (partial)
BPC2_A05	33	AE016764	34	100	Escherichia coli CFT073 section 10 of 18 of the complete genome
BPC2_C06	38	AF047646	696	96	Uncultured eubacterium TRB82 16S rRNA, partial
BPC2_F05	43	AF523950	628	93	Uncultured bacterium clone FW93 16S rRNA, partial
BPC2_F06	44	AF529115	575	91	Uncultured alpha proteobacterium clone FTLM116 16S rRNA
BPC2_E07	57	AF104274	65	91	Uncultured bacterium Benz-76 16S rRNA, partial
BPC2_E08	58	AJ252655	628	97	Agricultural soil bacterium clone SC-I-77, 16S rRNA gene (partial)
BPC2_F07	59	AF234690	537	95	Uncultured sludge bacterium H39 16S rRNA, partial
BPC2_E09	73	AJ534634	670	95	Uncultured Acidobacterium group bacterium partial 16S rRNA
BPC2_H10	80	AF427466	36	92	Symbiodinium sp. clade_A 18S rRNA, partial;
BPC2_A12	82	AJ518464	95	90	Unidentified bacterium partial 16S rRNA clone Neu4P3-86
BPC2_D12	88	AJ532711	678	94	Uncultured gamma proteobacterium partial 16S rRNA clone

Table A-IV 49. Peru Creek Summer: ARB Divisions

ARB Name	RFLP Type	Number of RFLPs	ARB Division	ARB Subdivision		Bases
BPC3_A01	1	1	bacterial environmental samples			804
BPC3_A02	2	1	Acidobacteria	environmental		830
BPC3_B01	3	1	OP10	environmental		841
BPC3_C01	5	1	Proteobacteria	Alpha	environmental	865
BPC3_C02	6	1	Proteobacteria	Beta	Burkholderiales	853
BPC3_D01	7	1	ARD1	environmental		805
BPC3_D02	8	1	Bacteroidetes	Saprospiraceae	environmental	784
BPC3_E01	9	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	531
BPC3_E02	10	1	Bacteroidetes	Bacteroidales	environmental	816
BPC3_F01	11	1	Proteobacteria	Beta	environmental	848
BPC3_F02	12	1	Proteobacteria	Beta	Burkholderiales	842
BPC3_G01	13	1	Verrucomicrobia	VER-3	environmental	730
BPC3_G02	14	1	Proteobacteria	Gamma	Environ. clone group	841
BPC3_H01	15	1	Proteobacteria	Beta	Burkholderiales	821
BPC3_H02	16	1	Proteobacteria	Gamma	Environ. clone group	849
BPC3_A04	18	1	Cyanobacteria	environmental		823
BPC3_B03	19	1	Proteobacteria	Beta	Burkholderiales	846
BPC3_B04	20	1	Proteobacteria	Gamma	Xanthomonadales	846
BPC3_C03	21	1	ARD2	environmental		836
BPC3_C04	22	1	Proteobacteria	Gamma	environmental	830
BPC3_D03	23	1	Proteobacteria	Gamma	Environ. clone group	785
BPC3_D04	24	1	Planctomycetes	Isosphaera	environmental	835
BPC3_E03	25	1	Acidobacteria	Acidobacteria-1	Acap group	862
BPC3_E04	26	1	Bacteroidetes	Sphingobacteriaceae	environmental	800
BPC3_F04	28	1	Acidobacteria	Acidobacteria-1	Acap group	817
BPC3_G03	29	1	ARD1	environmental		828
BPC3_G04	30	1	Proteobacteria	Beta	Burkholderiales	612
BPC3_H04	32	1	Acidobacteria	Acidobacteria-3	environmental	389
BPC3_A05	33	1	Acidobacteria	Acidobacteria-1	environmental	842
BPC3_B05	35	1	ARD2	environmental		774
BPC3_B06	36	1	Proteobacteria	Alpha	DA122 soil group	863
BPC3_C06	38	1	Proteobacteria	Alpha	Acetobacteraceae	849
BPC3_E05	41	1	Acidobacteria	environmental		848
BPC3_E06	42	1	Acidobacteria	ARD environmental group		607
BPC3_F05	43	1	Acidobacteria	Acidobacteria-1	Acap group	863
BPC3_F06	44	1	Bacteroidetes	Sphingobacteriaceae	environmental	794
BPC3_G05	45	1	Verrucomicrobia	VER-2	environmental	844
BPC3_G06	46	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	859
BPC3_H05	47	1	Proteobacteria	Delta	Myxococcales	799
BPC3_H06	48	1	Proteobacteria	Alpha	environmental	844
BPC3_A07	49	1	Proteobacteria	Alpha	environmental	855
BPC3_A08	50	1	Proteobacteria	TM6	environmental	865
BPC3_B08	52	1	bacterial environmental samples			801
BPC3_C07	53	1	OP10	environmental		604

Table A-IV 49. Peru Creek Summer: ARB Divisions, continued

ARB Name	RFLP Type	Number of RFLPs	ARB Division	ARB Subdivision		Bases
BPC3_C08	54	1	Proteobacteria	Alpha	Acetobacteraceae	852
BPC3_D08	56	1	Proteobacteria	Gamma	Environ. clone group	632
BPC3_E07	57	1	Proteobacteria	Beta	environmental	725
BPC3_E08	58	1	Acidobacteria	Acidobacteria-1	Acap group	771
BPC3_F08	60	1	OP3	environmental		858
BPC3_G08	62	1	OP10	environmental		830
BPC3_H07	63	1	Chlorobi	environmental		841
BPC3_H08	64	1	Chloroflexi	ARD environmental group		822
BPC3_A09	65	1	Proteobacteria	Beta	environmental	827
BPC3_A10	66	1	ARD1	environmental		822
BPC3_B09	67	1	OP10	environmental		853
BPC3_B10	68	1	Proteobacteria	Beta	Burkholderiales	852
BPC3_C09	69	1	Proteobacteria	Gamma	Environ. clone group	817
BPC3_C10	70	1	Proteobacteria	Beta	Burkholderiales	846
BPC3_D09	71	1	Proteobacteria	Beta	environmental	813
BPC3_D10	72	1	ARD1	environmental		802
BPC3_E09	73	1	Bacteroidetes	Saprospiraceae	environmental	866
BPC3_E10	74	1	Acidobacteria	Acidobacteria-1	environmental	845
BPC3_F09	75	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	861
BPC3_F10	76	1	Proteobacteria	Alpha	Bradyrhizobiaceae	846
BPC3_G09	77	1	Proteobacteria	Beta	environmental	839
BPC3_A11	81	1	Proteobacteria	Alpha	Bradyrhizobiaceae	788
BPC3_A12	82	1	ARD1	environmental		822
BPC3_B11	83	1	Proteobacteria	Gamma	Environ. clone group	839
BPC3_C11	85	1	Proteobacteria	Gamma	Environ. clone group	840
BPC3_C12	86	1	Acidobacteria	Acidobacteria-1	environmental	862
BPC3_D11	87	1	Acidobacteria	ARD environmental group		844
BPC3_E11	89	1	Proteobacteria	Gamma	Environ. clone group	839
BPC3_E12	90	1	Chloroflexi	ARD environmental group		819
BPC3_F11	91	1	ARD1	environmental		855
BPC3_G11	93	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	835
BPC3_G12	94	1	Bacteroidetes	Saprospiraceae	environmental	830
BPC3_H11	95	1	Chloroflexi	ARD environmental group		798
BPC3_H12	96	1	Acidobacteria	ARD environmental group		837

Table A-IV 50. Peru Creek Summer: Best BLAST Matches

ARB Name	Assession Number	Bit Score	% ID	Best Blast Match
BPC3_A01	AF428801	686	93	Uncultured bacterium clone CR98-5-71 16S rRNA,
BPC3_A02	AJ292580	775	97	uncultured eubacterium WD244 partial 16S rRNA clone WD244
BPC3_B01	AJ316618	716	96	Thiobacillus plumbophilus 16S rRNA strain DSM 6690
BPC3_C01	Y12596	1394	96	Uncultured bacterium DA111 16S rRNA gene
BPC3_C02	AY050592	1392	95	Uncultured bacterium clone GOUTB4 16S rRNA, partial
BPC3_D01	AJ519409	1413	97	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC3_D02	AY188323	1324	96	Uncultured bacterium clone KD6-86 16S rRNA, partial
BPC3_E01	AJ534627	749	95	Uncultured proteobacterium partial 16S rRNA clone JG36-TzT-192
BPC3_E02	AF351234	916	89	Uncultured CFB group bacterium clone 8-1 16S rRNA,
BPC3_F01	AF351236	842	99	Uncultured beta proteobacterium clone 8-5 16S rRNA,
BPC3_F02	AF407387	1247	94	Uncultured bacterium clone RB7C10 16S rRNA, partial
BPC3_G01	AF523996	981	94	Uncultured bacterium clone FW49 16S rRNA, partial
BPC3_G02	AJ292673	1612	99	uncultured eubacterium WD260 partial 16S rRNA clone WD260
BPC3_H01	AF407387	1507	98	Uncultured bacterium clone RB7C10 16S rRNA, partial
BPC3_H02	AY050601	850	96	Uncultured bacterium clone GOUTB18 16S rRNA, partial
BPC3_A04	AF076163	1346	95	Uncultured Antarctic bacterium LB3-47 16S rRNA, partial
BPC3_B03	AJ536880	1300	95	Uncultured bacterium partial 16S rRNA clone JG30-KF-AS41
BPC3_B04	AB074598	1219	93	Uncultured gamma proteobacterium gene for 16S rRNA, partial,
BPC3_C03	AJ292684	763	96	uncultured eubacterium WD272 partial 16S rRNA clone WD272
BPC3_C04	AY050601	1108	94	Uncultured bacterium clone GOUTB18 16S rRNA, partial
BPC3_D03	AJ292673	1453	98	uncultured eubacterium WD260 partial 16S rRNA clone WD260
BPC3_D04	X81958	1140	93	Isophaera sp. partial 16S rRNA gene (Schlesner 666)
BPC3_E03	AJ292583	1372	95	uncultured eubacterium WD257 partial 16S rRNA clone WD257
BPC3_E04	AF059758	1342	95	Uncultured eubacterium DgEPI2 16S rRNA, partial
BPC3_F04	AJ292578	1487	98	uncultured eubacterium WD228 partial 16S rRNA clone WD228
BPC3_G03	AJ519409	1437	97	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC3_G04	AF414586	1047	98	Uncultured bacterium clone P3OU-53 16S rRNA, partial
BPC3_H04	AJ519380	521	92	Uncultured Holophaga sp. partial 16S rRNA clone JG37-AG-74
BPC3_A05	AF200699	1552	98	Uncultured Acidobacterium UA3 16S rRNA, partial
BPC3_B05	AJ292684	1009	91	uncultured eubacterium WD272 partial 16S rRNA clone WD272
BPC3_B06	AJ292606	1336	94	uncultured eubacterium WD295 partial 16S rRNA clone WD295
BPC3_C06	AF523948	1447	96	Uncultured bacterium clone FW138 16S rRNA, partial
BPC3_E05	AJ292579	1477	97	uncultured eubacterium WD243 partial 16S rRNA clone WD243
BPC3_E06	AJ292580	1039	96	uncultured eubacterium WD244 partial 16S rRNA clone WD244
BPC3_F05	AJ292579	1235	94	uncultured eubacterium WD243 partial 16S rRNA clone WD243
BPC3_F06	AF059758	1400	96	Uncultured eubacterium DgEPI2 16S rRNA, partial
BPC3_G05	U51864	1261	94	Unidentified eubacterium EA25 16S rRNA, partial
BPC3_G06	AF514854	1608	98	Haslea salstonica 16S rRNA, partial;
BPC3_H05	AY187312	1029	94	Uncultured bacterium clone csbio160363 16S rRNA,
BPC3_H06	AJ458486	1312	95	Methylosinus sporium partial 16S rRNA strain 8
BPC3_A07	AJ536879	1138	99	Uncultured bacterium partial 16S rRNA clone JG30-KF-AS89
BPC3_A08	AF255637	1580	98	Uncultured bacterium clone Ebpr6 16S rRNA, partial
BPC3_B08	AY093470	1243	94	Uncultured bacterium clone MB-B2-105 16S rRNA, partial
BPC3_C07	AJ536891	722	90	Uncultured bacterium partial 16S rRNA clone JG30-KF-CM64
BPC3_C08	AJ292606	1548	98	uncultured eubacterium WD295 partial 16S rRNA clone WD295

Table A-IV 50. Peru Creek Summer: Best BLAST Matches

ARB Name	Accession Number	Bit Score	% ID	Best Blast Match
BPC3_D08	AJ292673	1168	98	uncultured eubacterium WD260 partial 16S rRNA clone WD260
BPC3_E07	AJ316618	1257	97	Thiobacillus plumbophilus 16S rRNA strain DSM 6690
BPC3_E08	AJ292579	1350	97	uncultured eubacterium WD243 partial 16S rRNA clone WD243
BPC3_F08	AF422606	733	91	Uncultured bacterium clone t036 16S rRNA, partial
BPC3_G08	AJ009456	438	89	uncultured bacterium SJA-22 16S rRNA clone SJA-22
BPC3_H07	AJ536877	1031	90	Uncultured bacterium partial 16S rRNA clone JG30a-KF-39
BPC3_H08	AJ536876	1168	93	Uncultured bacterium partial 16S rRNA clone JG30a-KF-32
BPC3_A09	AJ536880	1616	99	Uncultured bacterium partial 16S rRNA clone JG30-KF-AS41
BPC3_A10	AJ519409	1382	97	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC3_B09	AJ244807	1017	95	Gram-positive bacteria SOGA31 partial 16S rRNA gene
BPC3_B10	AF414586	1453	97	Uncultured bacterium clone P3OU-53 16S rRNA, partial
BPC3_C09	AJ292673	1324	97	uncultured eubacterium WD260 partial 16S rRNA clone WD260
BPC3_C10	AF289156	1594	98	Uncultured beta proteobacterium clone PRD01a008B 16S rRNA
BPC3_D09	AF351236	1176	94	Uncultured beta proteobacterium clone 8-5 16S rRNA,
BPC3_D10	AJ519409	1409	97	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC3_E09	AY188323	1457	96	Uncultured bacterium clone KD6-86 16S rRNA, partial
BPC3_E10	AF498692	1404	96	Bacterium Ellin310 16S rRNA, partial
BPC3_F09	AF418975	1485	97	Uncultured diatom clone HT2F1 16S rRNA, partial
BPC3_F10	Z94805	1606	99	Bradyrhizobium genosp. P 16S rRNA gene
BPC3_G09	AJ536880	1618	99	Uncultured bacterium partial 16S rRNA clone JG30-KF-AS41
BPC3_A11	Z94805	1400	97	Bradyrhizobium genosp. P 16S rRNA gene
BPC3_A12	AJ519409	1338	96	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC3_B11	Z94805	955	97	Bradyrhizobium genosp. P 16S rRNA gene
BPC3_C11	AJ292673	1620	99	uncultured eubacterium WD260 partial 16S rRNA clone WD260
BPC3_C12	AF200699	1394	96	Uncultured Acidobacterium UA3 16S rRNA, partial
BPC3_D11	AJ536864	1562	98	Uncultured Holophaga sp. partial 16S rRNA clone JG30-KF-C37
BPC3_E11	AJ534627	1235	94	Uncultured proteobacterium partial 16S rRNA clone JG36-TzT-192
BPC3_E12	AJ536876	912	91	Uncultured bacterium partial 16S rRNA clone JG30a-KF-32
BPC3_F11	AJ519409	1507	98	Uncultured bacterium partial 16S rRNA clone JG37-AG-138
BPC3_G11	AF523902	1536	99	Uncultured bacterium clone RCP2-13 16S rRNA, partial
BPC3_G12	AY188323	1394	96	Uncultured bacterium clone KD6-86 16S rRNA, partial
BPC3_H11	AJ536876	1328	96	Uncultured bacterium partial 16S rRNA clone JG30a-KF-32
BPC3_H12	AF465650	1509	97	Uncultured Acidobacterium group bacterium YNPRH2B 16S rRNA

Table A-IV 37. Pennsylvania Mine Snowmelt: ARB Divisions

ARB Name	RFLP type	# of RFLPs	ARB Division	ARB Subdivision		Bases
BPM2_A01	1	1	Actinobacteria	Acidimicrobidae	acidimicrobium	819
BPM2_H01	2	2	Proteobacteria	Alpha	Acetobacteraceae	809
BPM2_B02	3	7	Proteobacteria	Gamma	Xanthomonadales	814
BPM2_C01	4	23	Proteobacteria	Beta	Burkholderiales	812
BPM2_C02	5	2	Cyanobacteria	chloroplasts	Euglena chloroplasts	800
BPM2_D01	6	1	Bacteroidetes	Flavobacteriales	Sporocytophaga	822
BPM2_E01	7	1	Acidobacteria	Acidobacteria-1	Acap group	782
BPM2_F01	8	6	Proteobacteria	Alpha	environmental	830
BPM2_G01	9	1	Actinobacteria	Rubrobacteridae	MC4	814
BPM2_H02	10	1	Acidobacteria	Acidobacteria-1	Acap group	810
BPM2_A03	11	1	Proteobacteria	Beta	Burkholderiales	807
BPM2_A04	12	1	Proteobacteria	Beta	Burkholderiales	812
BPM2_B03	13	2	Bacteroidetes	Flexibacteraceae	environmental	798
BPM2_C03	14	1	Proteobacteria	Alpha	environmental	778
BPM2_D03	15	2	Proteobacteria	Alpha	environmental	652
BPM2_E03	16	1	Chloroflexi	ARD clone		793
BPM2_F03	18	1	Proteobacteria	Delta	environmental	822
BPM2_H03	19	2	Firmicutes	alicyclobacillaceae		810
BPM2_A05	20	1	Cyanobacteria	chloroplasts	Euglena Chloroplasts	824
BPM2_A06	21	3	Actinobacteria	Micrococcaceae	Arthrobacter	832
BPM2_C06	22	1	Proteobacteria	Alpha	Acetobacteraceae	827
BPM2_D06	23	1	Proteobacteria	Alpha	Acetobacteraceae	834
BPM2_E06	24	2	Verrucomicrobia	VER-1	environmental	813
BPM2_F06	25	1	Acidobacteria	Acidobacteria-1	Acap group	808
BPM2_G05	26	2	Planctomycetes	Gemmata	environmental	815
BPM2_H05	27	1	Proteobacteria	TM6	environmental	812
BPM2_H06	28	1	Actinobacteria	Microbacteriaceae	environmental	826
BPM2_B08	29	1	Actinobacteria	Intrasporangiaaceae	environmental	807
BPM2_D07	30	2	ARD2	environmental		817
BPM2_F08	31	1	Acidobacteria	Acidobacteria-1	Acap group	832
BPM2_G08	32	1	Chloroflexi	ARD clone		793
BPM2_H07	33	2	Proteobacteria	Beta	Burkholderiales	825
BPM2_A09	35	1	Acidobacteria	Acidobacteria-3	environmental	808
BPM2_A10	36	1	Actinobacteria	Corynebacterineae	Gordoniaceae	822
BPM2_B10	37	2	Proteobacteria	Gamma	Xanthomonadales	842
BPM2_D10	38	1	ARD1	environmental		806
BPM2_H10	39	1	Proteobacteria	Alpha	environmental	869
BPM2_B11	40	1	OP11	environmental		821
BPM2_C11	41	1	Proteobacteria	Gamma	Xanthomonadales	835
BPM2_C12	42	1	Proteobacteria	Gamma	Xanthomonadales	564
BPM2_E12	43	1	Firmicutes	bacilli	Planococcaceae	847
BPM2_G11	44	1	Proteobacteria	Delta	environmental	807
BPM2_G12	45	1	Proteobacteria	Gamma	Xanthomonadales	812
BPM2_H12	46	1	Proteobacteria	Gamma	Xanthomonadales	839
BPM2_B07	47	1	Proteobacteria	Gamma	Xanthomonadales	849